EFFICACY OF ORAL ALTRENOGEST FOR POSTPONING OVULATION IN THE MARE

A Thesis

by

SANDRA LEE MURRELL

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2003

Major Subject: Physiology of Reproduction

EFFICACY OF ORAL ALTRENOGEST FOR POSTPONING OVULATION IN THE MARE

A Thesis

by

SANDRA LEE MURRELL

Submitted to Texas A&M University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Approved as to style and content by:

Martha M. Vogelsang (Chair of Committee)	Paul G. Harms (Member)
Mark E. Westhusin (Member)	John W. McNeill (Head of Department)

August 2003

Major Subject: Physiology of Reproduction

ABSTRACT

Efficacy of Oral Altrenogest for Postponing Ovulation in the Mare.

(August 2003)

Sandra Lee Murrell, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Martha M. Vogelsang

The horse industry relies heavily on the breeding manager's ability to get a large number of mares bred each season. Therefore, it is beneficial to optimize use of the stallion and number of cycles per conception, both of which are related to the time of ovulation. Currently, methods exist to induce ovulation of a Graafian follicle; however there is no widely accepted method for delaying ovulation. Considering the main factors that lead to ovulation it could be hypothesized that the short-term administration of a progestin, such as altrenogest, would have the potential to postpone ovulation of a follicle that has reached ovulatory size. Twenty-six mares ranging in age from three to 23 years were paired by age and randomly assigned to a 2X4 blocked experiment. The factors were 1) stallion (Groups A-D) and 2) hormonal treatment (.044mg/kg BW altrenogest for 2 d) or control (Neobee M5 oil for 2 d). Hormonal administration for each mare was initiated upon detection of a follicle with a 35 mm or greater diameter. Mares were artificially inseminated starting on the first day of treatment and continuing every other day until ovulation was confirmed by ultrasonography. Blood samples were collected daily throughout the estrous

cycle and at 12 h intervals from the detection of a 35 mm follicle until three days post-ovulation. Samples were analyzed for luteinizing hormone using a double antibody RIA, for progesterone and estradiol using RIA kits (Coat-A-Count® and double antibody, DPC®).

Short-term altrenogest administration increased the number of days to ovulation ($P<0.05$). Mean days to ovulation from d 1 of treatment for the control versus treated mares were 3.15 d and 6.12 d, respectively. There was no difference ($P=0.65$) between control and treated groups with respect to size of follicle at the time of ovulation. Luteinizing hormone, progesterone and estradiol concentrations were analyzed during treatment until 3 d post-ovulation. Altrenogest treatment had no effect on LH, progesterone and estradiol concentrations as demonstrated by the lack of difference between control and treated mares ($P=0.27$, $P=0.56$, $P=0.67$). There was no difference in pregnancy rates ($P=0.62$) between the control 10/13 and treated 11/13 mares.

DEDICATION

I dedicate this thesis to my family. To Mama, for just being you; loving, understanding and the most patient person I have ever known. To Papa, for coming to help at the farm and yet being completely grossed out by what I was doing. To my big sis, Steff, for always being on the other end of the phone line no matter what time it was. To my brother Steve, for being just what you are, my brother. Last but not least to Sam and Siouxsie, my support group and my "kids".

ACKNOWLEDGEMENTS

I would like to thank Dr. Martha Vogelsang for never giving up on me and for harassing me to get my things done. Without that I probably would have never finished this endeavor. Thank you for taking me on as a graduate student. I appreciate all you have done for me, most especially for getting me my internship, because it was from that point on that I knew what I wanted to do when I "grew up". I would also like to thank my other committee members, Dr. Harms and Dr. Westhusin, for their knowledge, both in the classroom and with my project, and their patience with me in finishing this project.

Thanks definitely go to Dita and Deb, because you two are responsible for getting me into this. You wouldn't allow me to take the easy way out. Joann, my saving grace, if it were not for you and your help with EVERYTHING, most especially the lab work, I would have quit a million times. Also, thanks to you as a fellow graduate student, teaching assistant, office mate and roommate.

For all those who helped with my project, I would like to express my genuine appreciation. Beth Bass, the horse center crew and 685's, I hope I did not make that breeding season difficult with the restrictions of my project. Volunteers from my biology classes, thanks for your help with the data collection.

Much gratitude goes to those who helped so much with the lab work. Without their assistance the LH assay might never have been figured out. Dr. Roser and Lil Sibley, thank you for supplying the first antibody and for sending

numerous emails to help us figure out the problems with the assay. Dr. Parlow, thank you for supplying the standards. Lastly, Dr. Thompson, thank you for spending a spring break with us, I am truly grateful you would take the time to work with us.

Special thanks go to those who inspired me and taught me most of what I know about equine reproduction. Kelly Riccitelli, thanks for being such a strong influence. Stephen Vogelsang, thank you for taking me on as an intern. That is half a year of my life that I will not soon forget.

TABLE OF CONTENTS

CHAPTER Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

Management of the mare's estrous cycle, especially during the breeding season, is a major requirement for a breeding operation. Manipulation of estrus and time of ovulation are primary factors involved in controlling the cycle and play a key role in the success of a breeding program. Unlike other domestic species, mares have a long follicular phase that makes artificial control of estrus more difficult. Reasons for controlling time of ovulation are: a) space the breeding of a group of animals over a period of time to increase labor and management efficiency; b) breed a group of animals within a small window of time due to show schedules of either the mares or the stallion; c) schedule breeding of individual mares at predetermined times to help owners with board fees or for shipped semen; and d) synchronize donor and recipient mares in an embryo transfer program.

The use of artificial insemination (AI) enables the breeder to improve breeding management by optimizing use of the stallion, using a minimal number of breedings and decreasing the number of cycles per conception in some mares. The introduction of sperm into the female tract by AI must precede ovulation. Successful application of AI requires the ability to judge when ovulation will occur. If ovulation can be controlled, handling of mares is made

---

This thesis follows the style of the Journal of Animal Science.

easier by having to breed a mare only once. For these reasons, it would be beneficial to be able to postpone as well as induce ovulation of a mature follicle. Currently, several methods exist to induce rupture of follicle: an injection of human chorionic gonadotropin; GnRH analogue (Buserelin) or agonist (Gonadorelin) given as an implant or an injection (Ovuplant®, Deslorelin); or an injection of prostaglandin analogue (Fenprostelene). However, there is no regimen to delay ovulation of a mature follicle by a couple of days.

Ovulation occurs as a result of exposing a preovulatory follicle to high levels of luteinizing hormone (LH). At the beginning of the follicular phase, LH secretion increases due to the combined effects of estradiol-17β and progesterone on the hypothalamic-pituitary-gonadal axis. Positive feedback of estradiol-17β from the growing follicle on the hypothalamus increases GnRH secretion. Decline in the negative feedback of progesterone secretion from the corpus luteum on the hypothalamus further allows GnRH secretion to increase. Increasing GnRH secretion signals the adenohypophysis to switch from follicle stimulating hormone (FSH) secretion to LH secretion. Conversely, at the beginning of the luteal phase, LH secretion decreases due to a switch in the above mentioned feedback mechanisms. Estradiol levels peak and drop before ovulation, these low levels no longer provide positive feedback on the hypothalamus. After rupture of the follicle, progesterone production increases from the newly formed corpus luteum. Therefore, it would be reasonable to propose that since progesterone has a negative feedback on LH secretion, an

exogenous progestagen could delay the interval between selection of a predominant follicle and ovulation.

There is a commercially available oral synthetic progestin, altrenogest (Regumate®), developed and approved for use in controlling the estrous cycle in the mare. Long term altrenogest administration (10 to 15 days) has been shown to suppress estrus and ovulation by prolonging the luteal phase (Daels et al., 1996). However, limited studies are available regarding the effects of short-term altrenogest administration late in the follicular phase.

Objective

The objective of this study is to validate and define the ability of altrenogest administration during estrus to postpone ovulation of a mature follicle in the mare. The hypothesis to be tested is that mares given altrenogest will demonstrate a delay in ovulation versus controls. The expected results are that altrenogest treated mares will demonstrate a longer interval to ovulation, have lower LH concentrations and no difference in either progesterone or estradiol concentrations versus control mares. Additionally, pregnancy rates for treated versus control mares will be evaluated to provide credibility for the treatment regimen.

CHAPTER II

LITERATURE REVIEW

Dynamics of the Estrous Cycle

Follicular phase. The follicular phase, also known as estrus, varies in length from four to seven days in the mare (Blanchard et al., 1998). Estrus is accompanied by follicular growth, selection, maturation and then ovulation. Follicular growth results from a surge in follicle stimulating hormone (FSH) release from the adenohypophysis. Selection of a dominant follicle is due to decreasing levels of FSH and increasing levels of luteinizing hormone (LH) from the adenohypophysis (Samper, 2000). Systemic levels of FSH decrease while LH increases due to increasing estrogen production from the growing follicles. Final maturation of the follicle is accomplished by a switch in gonadotropin dependency of the mature follicle from FSH to LH. There is a marked decrease in the number of FSH receptors on the granulosa cells along with acquisition of LH receptors (Samper, 2000). Ovulation occurs approximately 48 hours before the end of estrus in mares (Ginther, 1992; Mckinnon and Voss, 1993). The high concentration of LH or LH surge results in rupture of the follicle (ovulation).

Luteal phase. The luteal phase, also known as diestrus, accounts for the remainder of the estrous cycle. It is usually about 14 to 15 d in length, depending on the length of estrus which is more variable (Blanchard et al., 1998). Diestrus starts after ovulation by formation of the progesterone secreting

corpus luteum (CL). There is change of the follicular granulosa cells into luteal cells. Ovulation results in marked decrease of estrogen production. The combination of decreasing estrogen production and the increasing progesterone production leads to the decline in LH levels (Samper, 2000).

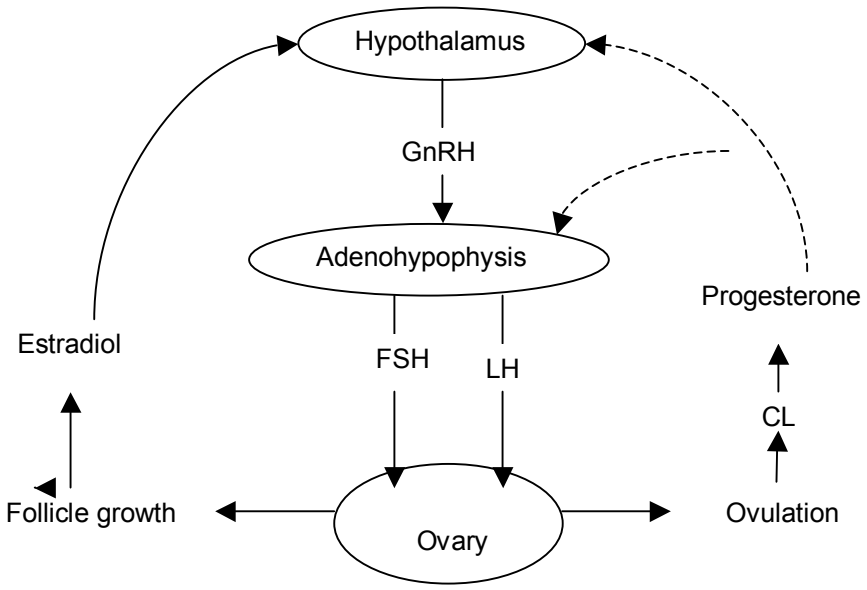

Figure 1. Schematic representation of the hormonal pathway of the hypothalamic-hypophyseal-gonadal axis during the estrous cycle. Solid lines indicate positive feedback. Dashed lines indicate negative feedback.

Hormonal Events of Ovulation

Luteinizing hormone. Ovulation occurs during the follicular phase of the estrous cycle and is the result of a cascade of events that is initiated by a surge of LH. The gonadotropin, LH, is necessary for final follicular growth, ovulation and formation of the corpus luteum (CL) (Guyton and Hall, 1996). The source of LH is gonadotroph cells of the adenohypophysis. The peptide hormone, GnRH, controls the circhoral (hourly) pattern of LH secretion from the adenohypophysis. GnRH is synthesized and released by neurosecretory cells located in the median eminence of the hypothalamus (Ginther, 1992) and reaches the adenohypophysis by the hypothalamic-hypophyseal portal system (refer to Figure 1). Episodic secretion along with fluctuations in frequency and amplitude of GnRH secretion are important in generating the hormonal changes during the cycle (Guyton and Hall, 1996). More frequent pulses late in the follicular phase lead to the LH surge (Turner and Irvine, 1991). Gonadotropin patterns of the mare are considerably different compared with other mammals. Most mammals exhibit a short-lived surge of gonadotropins 12 to 24 hours prior to ovulation; however, the mare has a prolonged rise and decline of LH which occurs over a period of about 10 days (Ginther, 1992). During mid-diestrus, mean LH values in mares are low and remain low until beginning to rise a few days before the onset of estrus. Luteinizing hormone concentrations increase progressively after the onset of estrus to peak one to two days after ovulation (Ginther, 1992;

Irvine and Alexander, 1997). Concentrations then decrease progressively over the next four to six days to return to low diestrous values (Ginther, 1992).

Estradiol. LH secretion is regulated by complex interactions among gonadal steroid hormone secretions, neuromodulators and neurotransmitters (Alexander et al., 1995). The ovarian hormone, estrogen (E), is responsible for behavioral estrus in mares and facilitates the LH surge (Ginther, 1992). It has the preeminent role of setting the neural trigger for the preovulatory gonadotropin surge (Mahesh and Brann, 1992). Estradiol is known for inducing an ovulatory-like surge of LH in females of numerous species (Alexander et al., 1995). Secretion of estradiol-17β, the primary estrogen produced, occurs from the granulosa cells of maturing, or Graafian, follicles. There is a steady increase of estradiol-17β, at the onset of estrus, with its peak occurring 12 to 27 h prior to ovulation (Ginther, 1992). Estradiol modulates LH by a long feedback loop to the hypothalamus and pituitary (Mahesh and Brann, 1992). Increasing estradiol-17β concentration has two modes of action for increasing LH secretions that will lead to ovulation. First, there is a direct increase in pulsatile secretions of GnRH from the hypothalamus. The second mode is by increasing the sensitivity of the anterior pituitary cells to GnRH by increasing the number of receptors located on the gonadotropes (Knobil and Neill, 1999). One possible way to postpone ovulation, therefore, could be to suppress estradiol-17β. However, the physiological actions of estrogen are exhibition of behavioral estrus, relaxation of the cervix, increased diameter of the cervical canal, increased secretion of

oviductal fluid and increased production of fluid with high lubricating properties from both the cervix and vagina (Knobil and Neill, 1999). All of these are required for preparing the right environment for the sperm and ovum before fertilization and for early embryo development (Ginther, 1992).

Progesterone. The ovarian hormone, progesterone (P), is considered the hormone of pregnancy because its presence results in quiescence of the smooth muscle of the uterus and prevents behavioral and physiologic signs of estrus. The CL secretes P during the luteal phase of the estrous cycle resulting in the diestrus between consecutive ovulations. Progesterone exerts a powerful negative feedback on the release of LH (Garcia et al., 1979; Allen et al., 1980) from the pituitary and hypothalamus. Luteinizing hormone concentrations do not start to rise until after regression of the CL (Garcia and Ginther, 1978; Evans, 1982). Sharp and Grubaugh (1987) showed that the hypothalamic release of GnRH is slowed at the end of estrus during the time that P is increasing. Progesterone decreases the pulse frequency of GnRH, thereby decreasing the signal for the release of LH (Karsch, 1987). Progesterone concentrations during estrus are < 2ng/ml and do not have a negative control over LH, thus allowing the cascade of hormones leading to the ovulatory surge of LH. Progesterone, in the mare, alone does not activate tonic opioid inhibition of LH but modulates the effect of estrogens (Aurich et al., 1995). Progesterone receptors on granulosa cells of several species (Schreiber and Erickson, 1979; Jacobs and Smith, 1980; and Jacobs et al., 1980) are important in that they allow P to augment the ability

of FSH to stimulate pregnenolone synthesis. Pregnenolone is used to create P instead of allowing the cholesterol to be converted into estrogens (Fanjul et al., 1983). At the level of the granulosa cell, P also provides a negative feedback on the aromatization of androgens to estradiol-17β. The administration of P always suppresses estradiol and its feedback (Knobil and Neill, 1999). Therefore, the administration of a progestin during estrus could have the potential to halt the growth, maturation and eventual ovulation of a follicle.

Hypothalamus

The hypothalamus is medially located and the most ventral portion of the third ventricle. It forms the lower parts of the lateral walls of the third ventricle. Its anterior boundary is the optic chiasm. Its posterior boundary is defined by the mammillary bodies. It is separated from the temporal lobes, laterally, by the hypothalamic sulci. The base of the hypothalamus is the *tuber cinereum*. The median eminence is the central part of the *tuber cinereum*.

Regulation of GnRH secretion. There is a complex interplay of neurotransmitters and the neurons that secrete GnRH. The gonadal steroids (E and P) are the primary regulators of GnRH secretion. Since the GnRH neurons do not possess steroid receptors, the gonadal steroids must act through other neurons which do contain steroid receptors. The presence of monoamine, peptide or amino acid-containing afferent fibers has been documented on GnRH neurons, mainly in rodents and sheep (Caldani et al., 1993). Afferent fibers

conduct inward and so those neurotransmitter afferents would be going toward the GnRH neurons.

The involved monoamines are catecholamines (dopamine and noradrenaline) and indolamine (serotonin). Noradrenaline suppresses the LH pulses in ovariectomized rats. In contrast, noradrenaline triggers LH secretion when injected into estradiol-treated ovariectomized rats. And, noradrenaline is involved in the mechanism which triggers the preovulatory surge of LH in conjunction with adrenaline (Knobil and Neil, 1999).

Dopamine inhibits the pulsatile release of LH in ovariectomized rats. It also contributes to the negative feedback of estradiol and therefore is believed to play a role in prepubertal animals. The stimulatory and inhibitory roles of dopamine have not been attributed to any specific parts of the brain (Caldani et al., 1993).

Serotonin induces inhibition of both the pulsatile and preovulatory release of LH. It also has stimulatory effects associated with the time of the preovulatory surge. During the anestrus period, in the ewe, serotonin is involved in the steroid independent-inhibition of the LH pulsatile release (Barraclough and Wise, 1982). In summary, the monoamines interact with steroids to alter GnRH secretion.

Various neuropeptides are able to alter GnRH secretion. Among them are opiates, β-endorphins and enkephalins. Of the aforementioned, opiates are probably the most important. Endogenous opioid peptides (EOPs) modulate LH

release by suppressing the GnRH pulse generator either in the hypothalamus and/or brain in several species (Behrens et al., 1993). Alterations in the steroid environment and stage of estrous cycle strongly influence the regulation of LH secretion by EOPs (Nanda et al., 1991). In the mare, evidence exists that EOPs are involved in the regulation of LH release. Opioids inhibit LH release in anovulatory mares during the non-breeding season and in cyclic mares during the luteal phase of the cycle (Aurich et al., 1995). Behrens et al. (1993) showed that naloxone, an opioid antagonist, did not lead to an increase in LH concentrations during the follicular phase in mares. Since opioids do not contribute to follicular phase dynamics they do not warrant consideration as a means of postponing ovulation.

The amino acid, gamma aminobutyric acid (GABA) is involved in many forms of inhibition in the central nervous system. GABA increases the interval between pulses of LH in rats, rabbits and sheep (Caldani et al., 1993). These different kinds of neurons can modulate physiology of the GnRH neurons directly or after integration of hormonal information has been received from the ovaries. As was previously mentioned, most of these afferents come from neurons which have the property to bind sexual steroids. So they also act through the steroids to alter the secretion of GnRH.

Adenohypophysis

The adenohypophysis (anterior pituitary) is suspended below the hypothalamus by the infundibulum and lies in a recess on the floor of the

cranium called the *sella turcica*. It is connected to the hypothalamus by the pituitary stalk, which arises from the median eminence (a midline, ventral projection from the *tuber cinereum*) and together they interact to direct the secretion of many different hormones. The adenohypophysis and the hypothalamus share a direct path of communication, the hypothalamic-hypophyseal portal system. This portal system directs the neurotransmitters released in the hypothalamus to the target cells of the adenohypophysis to mediate the release of specific hormones.

Gonadotropes. Gonadotropes are the target cells of the neurotransmitter GnRH. Gonadotropes are mainly localized within the pars distalis and pars tuberalis of the adenohypophysis. Kurosumi and Inoue (1986) described gonadotropes as follows: they contain large amounts of rough endoplasmic reticulum (RER) for their glycoprotein-producing role; they are considered granulated adenohypophyseal cells and contain a large number of Golgi apparati. The Golgi apparatus (GERL) is located in the juxtanuclear region near the center of the cell. The GERL consists of flattened membranous sacs (cisternae). There is a polarity associated with the poles of the Golgi. The *cis* face is the receiving side and is located near the RER. The *trans* face of the GERL is the shipping side. Overall, the GERL appears as a flattened stack of tubules with a slightly thicker membrane and a “fuzzy” substance (clathrin) coating the outside surface.

Gonadotroph secretion. Golgi apparati are especially extensive in cells specialized for secretion. Once the secretory protein, in this case a hormonal substance (LH or FSH), is formed within the RER, the protein is enfolded in transport vesicles that bud like bubbles from a specialized region called transitional endoplasmic reticulum. These specialized transport vesicles move the hormonal substance from the cisternae of the RER to the GERL. These vesicles attach and fuse with the cisternae of the adjacent GERL and convey their substance into the site of the secretory granule formation.

After stimulation and formation of the secretory granules, a gonadotrope must release its secretory substance. Mature secretory granules may leave the GERL on the trans face which gives rise to vesicles that are pinched off and move toward the cell surface to be discharged. Granules attach to the inside surface of the plasma membrane and fusion occurs at the contact point of the two membranes. After fusion, the granule slips out via exocytosis (Caldani et al., 1993). The hormone is released and travels to its target organ, the ovary.

Interactions of the Hypothalamus and Adenohypophysis

Generating the LH surge. The hypothalamus and adenohypophysis work together to produce the LH surge. The hormonal cascade begins at the hypothalamus. GnRH neurons are activated by a combination of internal and external stimuli as discussed previously. GnRH is then released by the neurons into the vascular system via the hypothalamic-hypophyseal portal system, which shunts blood directly to the adenohypophysis. GnRH binds to receptors on their

target cells the gonadotropes. The GnRH receptor is a special transmembrane receptor. When GnRH binds to the receptor, the end of the receptor protruding into the interior of the cell activates adenylate cyclase. Adenylate cyclase then converts ATP into cyclic adenosine monophosphate (cAMP), a second messenger, in the cytoplasm of the cell. The primary messenger is the hormone itself. The cAMP system as the second messenger is an important step of the pathway because it is necessary before further downstream events can occur. Cyclic AMP plays an important role in the regulation of cellular function (Greenstein, 1994). Activation of cAMP causes the activation of a family of control enzymes. Protein kinase is activated by cAMP which in turn causes a conformational change in proteins by the addition of a phosphate group. One of the functions of the second messenger system is the production and secretion of gonadotropin hormones. After activation, the gonadotropes release the hormone (FSH or LH), whichever was specified by the frequency and amplitude of the GnRH pulses, by exocytosis. The contents of the secretory vesicles are then released into the systemic bloodstream to seek their respective target tissues, the ovaries.

During diestrus, FSH release is due to the lower frequency release of GnRH. As a follicle begins to mature and release estradiol-17β, GnRH frequency begins to increase. This increase is due to the events discussed earlier which signal the GnRH neurons in the hypothalamus. The increase in the frequency of GnRH release is the modified signal to gonadotropes that they neet

to produce and secrete more LH and less FSH. In many species, ovulation occurs as a result of the increasing levels of LH (Shupnik, 1996).

Ovary

Two-cell theory. The "two-cell type, two gonadotropin theory" has been developed to explain follicular events of ovulation (Channing et al., 1978; and McNatty et al., 1980) (refer to figure 2). A wave of FSH initiates follicular recruitment and initial growth as was previously discussed. Around the end of diestrus, P levels decline which allows the intervals between GnRH pulses to increase. An increase in pulse frequency of GnRH signals an increase in LH production and release from gonadotropes (Karsch, 1987). The theca interna cells of the maturing follicle are directly affected by circulating LH. Androgen production begins as LH attaches to the receptors on the surface of the theca cells. The androgens then cross the basement membrane of the theca cells to reach the granulosa cells in the follicle. The granulosa cells are the second cell in the "two-cell" theory. The granulosa cell has receptors for FSH and LH, both gonadotropins affect androgen utilization by the cell. In the granulosa cell, androgens are aromatized to estradiol-17β to aid in further estrus stimulation. Here the path becomes more complex as estradiol-17β has several functions at the follicular level. One function is to act as a negative feedback control in the thecal cell, slowing down androgen production. This control mechanism is achieved by decreasing the responsiveness of LH receptors on the thecal cell. Another function of estradiol-17β, in conjunction with FSH, is to increase

responsiveness of the granulosa cell receptors. By increasing responsiveness of these receptors estradiol-17β can affect an increase in its own production to help prepare the follicle for ovulation. The increasing estradiol-17β exerts a positive feedback control at the level of the hypothalamus and the gonadotropes by increasing GnRH release and responsiveness of the GnRH receptors.

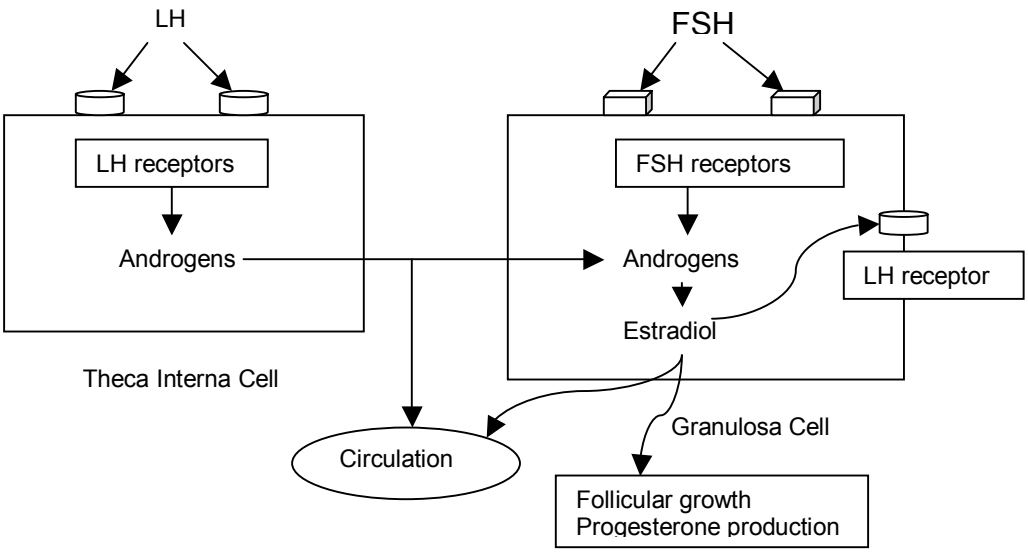

Figure 2. Schematic representation of the hormonal pathway between the thecal and granulosa cells in the ovary. Adapted from Greenstein (1996).

Altering Circulating Concentrations of LH

Circulating LH concentrations, in the mare, can be altered as demonstrated by Garcia and Ginther (1978). They demonstrated that estradiol-17β and P regulated plasma LH in ovariectomized mares and that P lowered LH concentrations when administered alone. When estradiol-17β was given alone, LH concentrations increased. Furthermore, when P and estradiol-17β were administered together there was a greater retarding effect on circulating concentrations of LH than when P was given alone. However, use of P and estradiol-17β showed no advantage for regulating LH in intact mares. Garcia and Ginther (1978) showed that P has a suppressing effect and estradiol-17β has a positive effect on circulating concentrations of LH in ovariectomized mares. This study demonstrated that P could be used to effectively decrease LH concentrations during estrus. However, it was not shown that influencing LH affected estrus or follicular growth.

The objective of this type of hormonal treatment is to delay not prevent ovulation. Therefore, effects on level of LH suppression must be considered in selecting the hormonal treatment to be utilized. Wiepz et al. (1988) compared altrenogest (2.2 mg/ml) and norgestomet (a progestin commonly used to synchronize estrus in cattle) and found similar concentrations of LH between the groups during the treatment period; however, concentrations of LH were consistently lower for the altrenogest group. When the treatment was discontinued, LH concentrations for the altrenogest-treated mares demonstrated

a twenty times greater increase than did the norgestomet-treated group, and the LH rise began immediately following the withdrawal of treatment. It is evident, therefore, that the effects of altrenogest can be fully reversed by discontinuing administration, thereby causing only a brief delay in LH secretion.

Influence of Progestagens on the Estrous Cycle

Naturally occurring and synthetic progestagens have proven effective for controlling estrus and ovulation in cycling females of other species (Loy and Swan, 1966). Studies on the effect of exogenous progestagens on estrus and ovulation in cycling and non-cycling mares have been reported. Loy and Swan (1966) found that estrus and ovulation could be suppressed in cycling mares by daily intramuscular injections of 100 to 400 mg of progesterone in oil. VanNiekerk et al. (1975) demonstrated that a normal, fertile estrus occurred within 3 to 4 days after cessation of daily injections of 100 to 125 mg of progesterone in oil in mares that were showing prolonged spring estrus. Injections were given for 7 to 9 days. Webel and Squires (1975) reported that the synthetic progestagen, allyl trenbolone, effectively blocked estrus and ovulation in cycling mares when given as oral doses of 0.176 to 0.440 mg/kg of body weight. Palmer (1979) was able to induce estrus and ovulation in anestrous pony mares in February and March. Pony mares were fed 20 mg of allyl trenbolone daily for 10 d at the end of a two-month artificial photoperiod. Squires et al. (1979) reported that mares fed 0.44 mg allyl trenbolone/kg BW for 12 d in March, commencing on the third day of physiological signs of estrus,

ovulated significantly ahead of control mares. Allen et al. (1980) reported that 10-15 d of Regumate® administered to anestrous mares resulted in behavioral estrus within eight days and ovulation within 18 d after treatment. Turner et al. (1981) observed that altrenogest inhibited follicular growth. After withdrawal, the inhibitory influence was released allowing increase in follicular size and ovulation of a smaller follicle than observed in control mares. Evans et al. (1982) demonstrated that exogenous P administered daily inhibited the increase of LH during estrus, with subsequent ovulation failure of pre-ovulatory follicles in cycling mares. Finally, Lofstedt and Patel (1989) showed that the ability of altrenogest to inhibit follicular growth was dependent on the stage of the estrous cycle when treatment began.

There have been studies that provide speculation relative to the efficiency of altrenogest administration to control ovulation. Lofstedt and Patel (1989) concluded that altrenogest was not effective for precise control of ovulation since 3 of 4 mares treated on the first three days of estrus ovulated by approximately day 10 of a 15 day treatment and had follicular growth greater than or equal to 35 mm. Loy and Swan (1966) found that neither 50 nor 100 mg IM of P in oil per day stopped estrus or blocked ovulation when treatment started on day one of estrus. Both of these studies utilized treatment regimens of 15 to 20 days of altrenogest administration and therefore do not provide information relating to short-term delay of ovulation.

Effect of Altrenogest on Pregnancy Rates

In development of a protocol for altering the estrous cycle, it is imperative that the chemical agent used does not negatively affect fertility. The use of altrenogest has shown no detrimental effects on conception rates when used in a protocol for estrus synchronization or for late transitional mares (Squires et al., 1979, 1983). Mares treated with oral allyl trenbolone (altrenogest) had pregnancy rates similar to untreated mares. Additionally, Allen et al. (1980) treated 38 Thoroughbred mares with allyl trenbolone and reported that 55% conceived when bred during the estrus after treatment.

Use of Progestins for Delaying Ovulation

Three studies using short-term administration of altrenogest, in an attempt to delay ovulation, after selection of a dominant follicle in the mare are reported in the literature. James et al. (1997) demonstrated that two days of altrenogest (0.044 mg/kg and 0.088 mg/kg body weight) administered at the beginning of standing estrus was successful for increasing the number of days to ovulation in mares with a 35+ mm follicle. Bruemmer et al. (2000) demonstrated, in mares treated with 0.088mg/kg BW of altrenogest for two days, a delayed interval to ovulation when compared to controls. However, in a second experiment that ran from June to August, there was no difference noted between altrenogest treated and control mares in days to ovulation. Neither study reported negative effect on fertility whether altrenogest was administered at the recommended dose (0.044 m/kg BW) or double the recommended dose

(0.088 mg/kg BW). James et al. (1997) reported period effects on days to ovulation during their study and therefore raised some questions on the effectiveness of Regumate to delay ovulation. Furthermore, James et al. (1997) examined LH concentration in treated versus control mares but did not evaluate P or E concentrations between treated and control mares. The current study was undertaken with those objectives in mind. Bruemmer et al. (2000) evaluated days to ovulation, LH and P concentrations between treated and control mares, however; their study was conducted after the current study.

CHAPTER III

EXPERIMENTAL PROCEDURES

Animals

Between February and July, twenty-six light body type mares ranging in from three to 23 years of age were used to study the efficacy of short-term altrenogest treatment for delaying ovulation with further evaluation of effects on fertility. Six mares were owned by the Texas A&M University's Department of Military Science, Parsons Mounted Cavalry (PMC). Twenty mares were owned by the Department of Animal Science at Texas A&M University. Four mares with foals, seven maiden mares and two open mares were maintained on native pastures at the TAMU Horse Center, College Station, TX. The six PMC and seven Department of Animal Science mares used for riding instruction were housed at their respective teaching facilities until May. At the end of the spring semester, these mares were moved to the TAMU Horse Center and remained on pasture for the duration of the project. All mares were fed for maintenance or early lactation based on NRC requirements (NRC, 1989).

Experimental design

Mares were assigned to one of four stallions, based on the need of the TAMU breeding program and then randomly assigned to either a treatment (0.044 mg/kg altrenogest) or a control (Neobee M5 oil, carrier oil for Regumate®) group (see Table 1). The progestin (altrenogest) administered was Regumate®

(chemical name: 17α-Allyl-17β-hydroxyestra-4,9,11-trien-3-one, Hoechst Roussel, Frankfurt, Germany). The manufacturer's recommended dose of Regumate® is 1 ml/110 lbs of body weight (0.044 mg/kg) because each ml of Regumate® contains 2.2 mg of altrenogest (0.22% or 2.2 mg/ml) in an oil solution (Neobee M5 oil). It was determined by James et al. (1997) and Bruemmer et al. (2000) that the recommended dose of Regumate® provided the most reliable results.

Table 1. Experimental design and assignment of mares to stallion and treatment.

	DundeeColonel	SkipAStake	Starman	EyesLegacy
Control	NuEnt (3) NuRey (4) CB (5) Merrily (5) Mandy (11)	MO (3) Skippy (6) Dixie (18)	FL (3) Mercury (4) Diane (11) Polly (23)	Starlet (3)
Treated	NBM (3) NNN (4) Snip (5) Binky (14) Ashley (13)	Maria (3) MM (6) Babe (20)	TF (3) Lady (5) CC (11) Sparticus (18)	PC (4)

Mares were teased daily by a stallion confined to a teasing cage located in the center of a paddock in which the mares were placed. Follicular activity was monitored by palpation and real time ultrasonography (Sonovet 600®, Universal Medical Systems, Inc., Bedford Hills, NY). Mares in both groups

received their first oral dose once a 35mm (an industry standard) or greater follicle was detected via ultrasound. Mares in the treatment group received altrenogest orally for two days and were artificially inseminated every other day, starting on the first day of treatment, until ovulation. Mares in the control group received Neobee M5 oil orally for two days and were artificially inseminated every other day starting on the first day of administration of carrier oil until ovulation. Detection of pregnancy was determined by ultrasound on day 12 post-ovulation and embryonic growth was assessed on day 14 and day 16. Prostaglandin (10 mg Lutalyse®, Upjohn, Kalamazoo, MI) was administered on day 16 to mares which were not part of the TAMU Department of Animal Science broodmare band. The injection of prostaglandin terminated pregnancies on the six PMC and seven Department of Animal Science mares and allowed them to return to their previous uses in riding instruction.

Semen Collection, Evaluation and Processing

All four stallions were known to be fertile based on past breeding histories. Semen was collected and processed in the same manner for each stallion and each insemination dose. During the collection, the ejaculate was filtered in line to remove the gel fraction. A small amount of the ejaculate was used to assess spermatozoal motility and concentration. Spermatozoal motility, a visual estimation of the percentage of spermatozoa that are progressively moving forward, was determined using a light microscope at 400 X magnification. Spermatozoal motility (%) was assessed by the same individual

throughout the study to minimize variation of this parameter. Concentration was determined using a Densimeter® (Model 534A, Animal Reproductive Systems, Chino, CA). Each insemination dose contained a minimum of 500 million motile sperm cells extended at a 1:1 ratio with E-Z Mixin®-"OF" or E-Z Mixin®-"CST" (Animal Reproduction Systems, Chino, CA) semen extender.

Sample Collection

Baseline blood samples were collected daily for each mare one estrous cycle prior to the treatment estrous cycle via jugular venipuncture. One blood sample per day is all that is necessary since the accumulative signal, not the pulses, are the focus of the project. During the time of administration of altrenogest or carrier oil and until three days post-ovulation, blood samples were collected twice a day (at 12 h intervals). In the mare, the LH peak is one to two days after ovulation so the last blood collection day was on the third day post-ovulation. Blood was collected into 10 ml sodium heparin vacutainers and cooled to 4 to 6°C. All vacutainers were centrifuged at 2500 x g for 15 min. The plasma was removed and aliquoted equally into three 2 ml microcentrifuge tubes, taken to the laboratory, frozen and stored at -20°C for later analysis. Plasma samples were analyzed for LH, progesterone and estradiol using radioimmunoassay (RIA) techniques.

Hormone Analyses

Plasma hormone concentrations were determined from daily baseline samples collected during the pre-treatment period. Beginning with the first day

of treatment through day three post-ovulation, analysis was conducted from samples collected every 12 h (twice daily) that were pooled into one composite sample for each day. All samples were assayed in duplicate. Plasma LH concentrations were also determined from the 12 h samples for comparison to the composite sample.

Plasma LH concentrations were determined using the Roser LH RIA protocol (Matteri et al., 1987) that had been previously validated (Lang et al., 1995) with several modifications. The first antibody (518B7, mouse anti-equine, Dr. J.F. Roser, UC Davis, CA) was diluted 1:30,000 in PBS-EDTA-normal mouse serum instead of RIA buffer. The second antibody (goat anti-mouse, Antibodies Inc., Davis, CA) was diluted 1:40 in PBS-EDTA instead of Polyethylene glycol (PEG). Standards (AFP5130A, eLH, Dr. A.F Parlow, Torrence, CA) and I^{125}-LH were diluted with PBS-Gelatin instead of RIA buffer. The use of Chloramine T for iodination in place of iodogen was another modification of the Roser protocol.

A 10 DG column (Bio-Rad, Hercules, CA) was conditioned with PBS and PBS-Gelatin. Approximately 7 μg of equine luteinizing hormone antigen (Dr. A.F. Parlow, Harvor-UCLA Medical Center, Torrance, CA, AFP-240580R6) was added to a reaction vial, followed by 1.0 mCi NaI^{125}, (NEN Life Sciences, #NEZ033A) and 10 μl Chloramine T at a dilution of 0.5 mg/ml. The reaction vial was mixed on ice for 15 sec and then transferred onto the conditioned column. The column was eluted with 10 ml of PBS-Gelatin and one ml fractions were

collected into labeled glass tubes. The fraction with the highest protein peak was further diluted to 15,000 cpm/100 μl. This stock I^{125}-LH was stored at 4°C for later use.

On the first day of the assay, 100 μl PBS-Gelatin, 100 μl standard or unknown sample, 200 μl first antibody and 100 μl of I^{125}-LH were added into the respective glass tubes and incubated at room temperature for 24 h. The following day, 200 μl of second antibody and 1 ml of cold 5% PEG were added to each tube. Tubes were centrifuged, decanted and counted for one minute. Sensitivity of the assay was 0.03 ng/ml. For 12 assays, the intra- and inter-assay coefficient of variation was 2.8% and 12.57%, respectively.

Plasma progesterone concentrations were determined using a single antibody RIA kit (Coat–a-Count®, DPC, Los Angeles, CA). Sensitivity of the assay was 0.02 ng/ml. For four assays, the intra- and inter-assay coefficient of variation was 6.12% and 4.11%, respectively.

Plasma estradiol concentrations were determined using a double antibody RIA kit (Double Antibody, DPC, Los Angeles, CA). Sensitivity of the assay was 0.46 pg/ml. For four assays, the intra- and inter-assay coefficient of variation was 5.97% and 8.7%, respectively.

Statistical Analysis

Data were subjected to analysis of variance using procedures appropriate for a randomized complete block (2 X 4) design using the general linear model (GLM) procedure of SAS (SAS Inst. Inc., Cary, NC). The main effects of mare,

treatment, days to ovulation as well as their interactions were analyzed. Mean follicle size at ovulation was evaluated using the student's t-test. Pregnancy rates were examined using Chi-Square procedures to determine reproductive efficiency (Hoshmand, 1988).

CHAPTER IV

RESULTS

Days to Ovulation

Number of days to ovulation for individual mares is shown in Table 2. Number of days to ovulation for treated and control groups is shown in Table 3. Similar to the findings of James et al. (1997) and Bruemmer et al. (2000), there was a difference ($p<.05$) between the treated and control groups in days to ovulation. Mean days to ovulation from day one of treatment for treated and control mares were 6.10 ± 3.41 d and 3.23 ± 2.0 d, respectively. To further examine the effect on days to ovulation, each group was divided into 3 periods, mares that ovulated in 1 to 3 d, 4 to 6 d and 7 to 14 d. Analysis of the 3 periods using an unpaired t test yielded a significant difference between groups ($p<.05$) (Table 4). Nine of the 13 control mares had ovulated within three days of starting treatment. Only two of the 13 treated mares had ovulated by day three. During the four to six days following treatment, three of the remaining four control mares and seven of the remaining eleven treated mares had ovulated. Finally, in the last period, days seven to 14 after starting treatment, the last control mare ovulated on day 8 and the remaining four treated mares ovulated. The number of mares that ovulated by d 2 and by d 6 during the treatment and control periods is shown in Table 5.

Table 2. Number of days to ovulation for individual mares treated with altrenogest vs controls.

Control		Treated	
Mare	**Day**	**Mare**	**Day**
Nu Ent	2	NBM	5.5
Nu Rey	3	NNN	12
CB	8	Snip	4
Merrily	3	Binky	5
Mandy	3.5	Ashley	6
MO	1	Maria	7
Skippy	.5	MM	4.5
Dixie	2	Babe	3
FL	2.5	TF	14
Mercury	3	Lady	7.5
Diane	5	CC	4
Polly	3	Sparticus	4
Starlet	5.5	PC	2.5

Table 3. Effect of altrenogest administration on days to ovulation for mares treated with altrenogest vs controls.

	n	Days to Ovulation
Control	13	3.23 ± 2.00^{a}
Treated	13	6.10 ± 3.41^{b}

Values are LSMeans $\pm$ SE

^{a,b} Columns lacking a common superscript differ ($p<.05$)

Table 4. Number of days to ovulation for mares treated with altrenogest vs controls separated into periods.

	Day 0-3	Day 4-6	Day 7-14
Control	9[a]	3[a]	1[a]
Treated	2[b]	7[b]	4[b]

[a,b] Columns lacking a common superscript differ ($p<.05$)

Table 5. Number of mares that ovulated by day 2 and day 6 after treatment with altrenogest vs controls.

		Number of mares that ovulated	
Group	n	Day 2	Day 6
Control	13	1	12
Treated	13	0	9

During the early part of this study, three maiden mares experienced a longer interval to ovulation, most likely the result of the spring transition. Furthermore, in each group (treated and control) there was one mare that developed a large follicle, underwent follicle regression and then switched to a secondary follicle on the other ovary which resulted in a prolonged interval to ovulation. Finally, one control mare ovulated a 35 mm follicle on the evening of the first day of treatment, thereby having an extremely short interval to ovulation.

Follicular Activity

Maximum follicular diameter for individual treated and control mares are shown in Table 6. Mean follicular diameters (mm) for treated and control groups were 43.62 $\pm$ 5.11 and 43.08 $\pm$ 6.75, respectively (Table 7). There was no difference ($p>.05$) between groups in mean follicular diameters at ovulation. This corroborates the findings of James et al. (1997).

Table 6. Follicular diameter (mm) for individual mares treated with altrenogest vs controls.

Control		**Treated**	
Nu Ent	45	NBM	42
Nu Rey	45	NNN	50
CB	52	Snip	47
Merrily	48	Binky	50
Mandy	45	Ashley	46
MO	37	Maria	43
Skippy	35	MM	48
Dixie	41	Babe	48
FL	41	TF	45
Mercury	47	Lady	52
Diane	57	CC	46
Polly	40	Sparticus	38
Starlet	49	PC	40

Table 7. Effect of altrenogest administration on follicular diameter at ovulation for treated vs controls.

Group	n	Follicular Diameter at Ovulation
Control	13	44.77 ± 1.44
Treated	13	45.77 ± 1.44

Values in column are LSMeans ± SE

Luteinizing Hormone

Luteinizing hormone concentrations were measured from daily pre-treatment plasma samples. From the beginning of treatment to three days post-ovulation, both 12 h samples and the pooled, composite samples were analyzed. Due to the variation of the interval between treatment and the number of days to ovulation, there were different numbers of samples for individual mares. Utilizing an unpaired t test, no significant difference was found between the mean of the 12 h samples and the pooled samples; therefore, the focus will be on the pooled samples. Mean composite peak LH concentration (ng/ml) for treated versus controls was 6.85 ± 0.54 and 10.10 ± 0.54, respectively. There was no effect of treatment on LH concentration for treated versus control mares. Figure 3 shows LH concentration of treated mares versus controls with day 0 as the day of ovulation. Although altrenogest did not significantly affect LH, the LH peak for treated mares was diminished as compared to the control group (Figure

3). This is similar to what James et al. (1997) reported. Bruemmer et al. (2000) also examined LH in one of their two studies. They reported a difference for treated mares in the amplitude of their LH peak versus controls.

Figure 4, shows the composite LH concentrations, with day 0 as the first day of treatment. The altrenogest treated mares do exhibit lower LH concentrations than the control mares on the two days of treatment, but then demonstrated increased LH within 24 hours after the second day of treatment. James et al. (1997) and Bruemmer et al. (2000) examined LH concentration around ovulation and not around the treatment period.

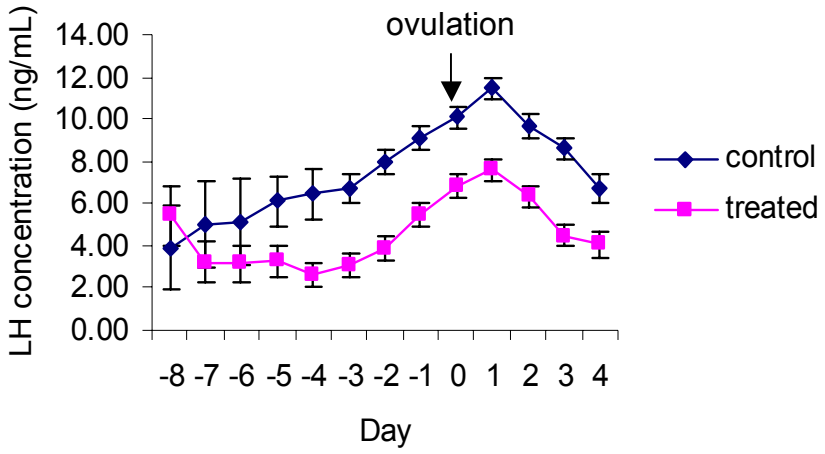

Figure 3. Composite LH concentration with day 0 as ovulation for mares treated with altrenogest vs controls.

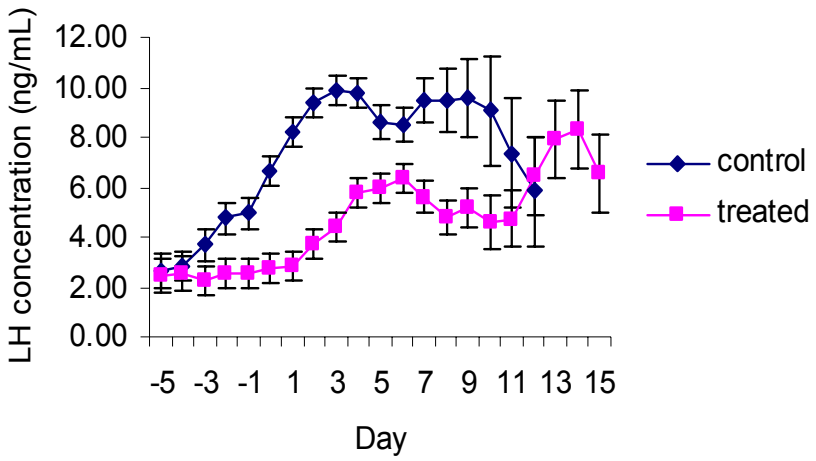

Figure 4. Composite LH concentration with d 0 as the first day of treatment for mares treated with altrenogest vs controls.

Progesterone

There was no effect of altrenogest treatment on progesterone concentrations for treated versus control mares (P=0.56). This is similar to the results of Bruemmer et al. (2000). Figure 5 illustrates the progesterone concentrations of altrenogest treated mares versus controls with day 0 as the day of ovulation. Progesterone levels during estrus, for both treated and control groups, are low (<1 ng/mL) two days prior to ovulation and begin to increase rapidly the two days following ovulation.

Figure 6 shows P concentrations of altrenogest treated mares versus controls with day 0 as the first day of treatment. Altrenogest treated mares exhibit a decline in P after the first day of treatment and levels do not start to increase until two days after the second day of treatment.

The progesterone levels are indicative of the native progestins released from the corpus luteum. The administration of Regumate® does not cross react with the RIA and therefore does not add to the values obtained by the assay.

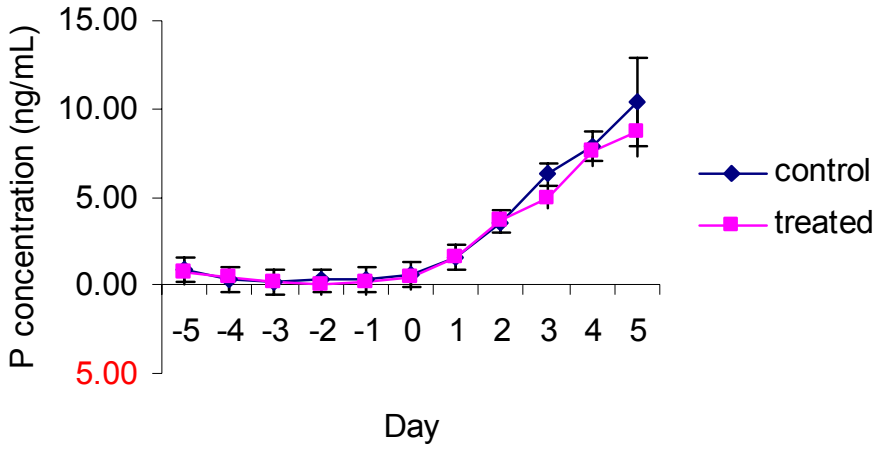

Figure 5. Progesterone concentration with day 0 as ovulation for mares treated with altrenogest vs controls.

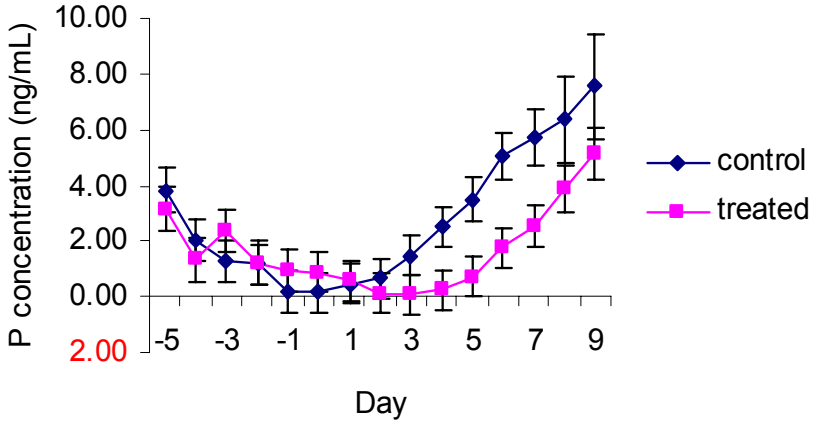

Figure 6. Progesterone concentration with day 0 as the first day of treatment for mares treated with altrenogest vs controls.

Estradiol

There was no effect of altrenogest treatment on estradiol concentrations for treated versus control mares (P=.67). Allen (1995) reported estradiol levels in barren and maiden mares to range from 5-20 pg/mL and in lactating mares to range from 150-200 pg/mL. The current study had four lactating mares, two control mares (300-600 pg/mL) and two treated mares (300-1400 pg/mL) with extremely high estradiol concentrations compared to the other mares.

Figure 7 shows estradiol concentrations with day 0 as the day of ovulation. Figure 8 shows estradiol concentration with day 0 as the day of ovulation for altrenogest treated mares versus control minus the four mares whose estradiol concentrations were outside the normally expected parameters. Both graphs illustrate the altrenogest treated and control mares have peak estradiol levels two days prior to ovulation which is characteristic of the preovulatory peak of estradiol. Estradiol levels then decline and remain low through ovulation and the end of the collection period. Figure 8 is more characteristic of the hormonal profile of E during this time of the estrous cycle. Figure 8 further shows that the treatment regimen did not affect the E curve.

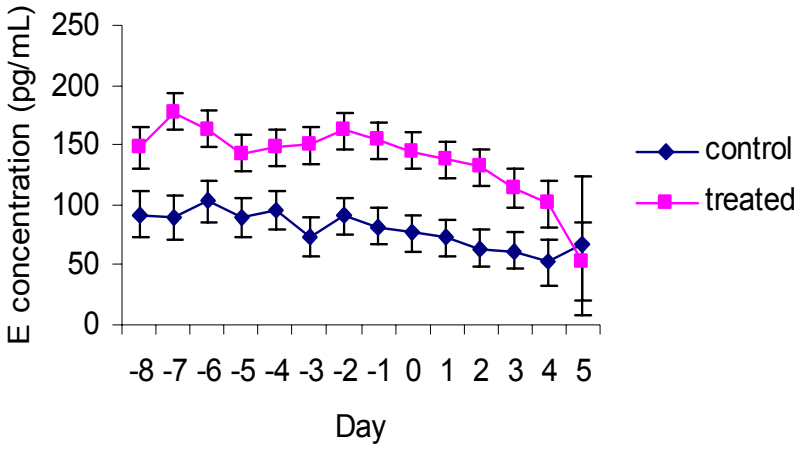

Figure 7. Estradiol concentration with day 0 as ovulation for mares treated with altrenogest vs controls.

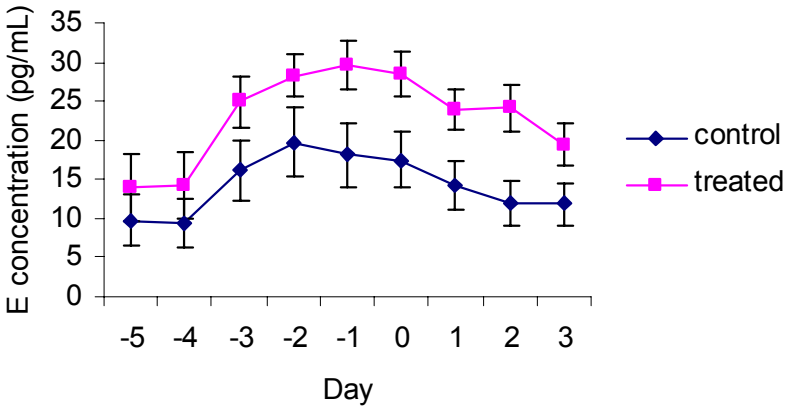

Figure 8. Estradiol concentration with day 0 as ovulation for mares treated with altrenogest vs controls (minus four mares whose E concentrations were outside normally expected parameters).

Figure 9 shows estradiol concentrations with day 0 as the first day of treatment. The altrenogest treated mares had overall higher concentrations of estradiol throughout the collection period and that can be noted in this figure. Figure 10 shows estradiol concentration with day 0 as the day of ovulation with the outlier mares removed from the mean. The figure includes the days with the most available data. Both the altrenogest treated and control mares demonstrate increasing estradiol concentration approaching the first day of treatment and continuing five days post treatment. Most of the control mares have ovulated by three days post treatment therefore the graph does not extend beyond this time frame.

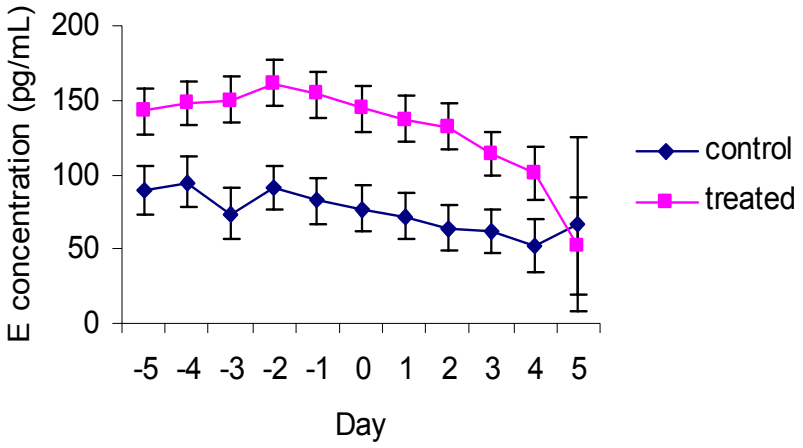

Figure 9. Estradiol concentration with day 0 as the first day of treatment for mares treated with altrenogest vs controls.

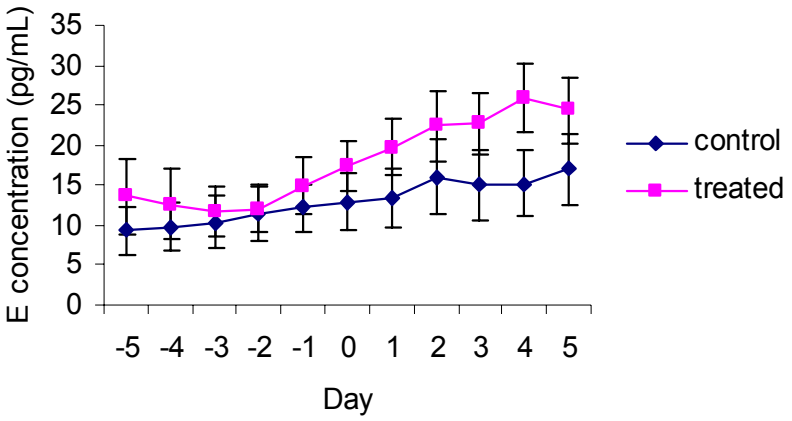

Figure 10. Estradiol concentration with day 0 as the first day of treatment for altrenogest treated mares vs controls (minus four mares whose E concentrations were outside normally expected parameters).

Pregnancy Rates

There was no effect of altrenogest treatment on pregnancy rates. This is in agreement with of James et al. (1997) and Bruemmer et al. (2000). Pregnancy rates were examined to determine the effect of altrenogest treatment, during estrus on the reproductive efficiency of the mares. The number of treated mares pregnant 12 days after ovulation was 11 out of 13, whereas the number of control mares that were pregnant 12 days after ovulation was 10 out of 13 (Table 9). Chi-square (0.23) was less than the critical value (3.84) concluding that the pregnancy rates were the same for altrenogest treated and control mares.

Table 8. Pregnancy rates for individual mares treated with altrenogest vs controls.

Control		Treated	
Nu Ent	P	NBM	P
Nu Rey	P	NNN	P
CB	P	Snip	P
Merrily	P	Binky	NP
Mandy	P	Ashley	P
MO	NP	Maria	P
Skippy	P	MM	P
Dixie	NP	Babe	NP
FL	P	TF	P
Mercury	P	Lady	P
Diane	P	CC	P
Polly	NP	Sparticus	P
Starlet	P	PC	P

Table 9. Pregnancy rates for mares treated with altrenogest versus controls.

		Number of Mares Pregnant	Pregnancy Rate (%)
Group	n		
Control	13	10	77
Treated	13	11	85

Embryonic development was also evaluated by ultrasonography on d14 and d16 for treated and control mares. Utilizing an unpaired t test on the data available there was no difference (P=.34, .90 and .69) in size of the embryonic vesicle on d12, d14 and d16, respectively, for altrenogest treated versus control mares (Table 10).

Table 10. Size of Embryonic Vesicle on d12, d14 and d16 after ovulation for mares treated with altrenogest versus controls.

Group	n	d 12	d 14	d 16
Control	10	10.67 ± 3.01	15.00 ± 3.16	25.16 ± 2.40
Treated	9	9.28 ± 2.05	15.33 ± 4.80	25.80 ± 2.77

CHAPTER V

GENERAL DISCUSSION

Days to Ovulation

Number of days to ovulation did show significant differences between the altrenogest treated mares and the control mares. Analysis of both groups that were divided into three periods also demonstrated a significant difference in days to ovulation between treated and control mares. Although the data are significant, there were two treated mares that ovulated by the third day after treatment and one control mare that ovulated after six days. Therefore, even though the treated mares had a significantly longer days to ovulation, the treatment still demonstrated some variability.

Palmer (1978) reported that the length of the follicular phase is dependent on the degree of follicular development based on circulating estrogen levels at the time of luteolysis. At the time of luteolysis, the length of the follicular phase and estrogen levels were negatively correlated. However, just prior to ovulation the length of the follicular phase and estrogen levels were positively correlated. Furthermore, Douglas and Ginther (1976) demonstrated that a single injection of prostaglandin significantly shortened the interovulatory interval. In the current study prostaglandin was administered to regress the corpus luteum after the baseline estrous cycle ovulation. Therefore, high estradiol levels at the time of either induced or natural luteolysis may have resulted in a decrease in the

number of days to ovulation for some mares in either the treated or control group.

Sirois et al. (1989) reported that the diameter of the preovulatory follicle at the time of progesterone decline was negatively correlated with the length of the follicular phase. Insufficient follicular data prevented analysis of this relationship for the current study. However, it could be a factor for either an increase or a decrease in the number of days to ovulation.

Follicular Activity

There was no difference between the altrenogest treated and control mares in size of follicle at ovulation. One factor that could have been avoided is when each mare was started on the project. The data would have been more representative if all of the mares had had a regular estrous cycle before the onset of the project. This study utilized mares in all phases of the breeding season; there were some maiden mares, spring transitional mares and mares going through foal heat. In order to get a better understanding of how altrenogest administration during estrus affects a mature follicle, larger numbers of mares from each parity group mentioned should be evaluated.

Bruemmer et al. (2000) reported that altrenogest treated mares had a greater incidence of follicular regression than did control mares. Those findings agree with Evans et al. (1982) which showed exogenous progesterone not only decreased LH concentrations but also resulted in failure of preovulatory follicles to ovulate. James et al. (1997) did not report on follicular regression. Every

mare in the current study had a cycle that resulted in an ovulation. However, there was one altrenogest treated and one control mare that did experience follicular regression in which they switched from a dominant follicle on one ovary to a dominant follicle on the other ovary. Both mares started with a large follicle on both ovaries. Examination of when the LH peak occurred corroborates that those mares did indeed switch dominant follicles and did not ovulate the first follicle. Another determination of ovulation in those mares was examination of progesterone levels. To differentiate ovulation and follicular regression plasma progesterone will increase and remain above 5ng/mL if an ovulation has occurred. Neither mare in question had an increase of progesterone during follicular regression. Results of the current study indicate that altrenogest treatment does not alter the final size a follicle will reach prior to ovulation. Instead it will only slow the growth necessary to reach the predetermined ovulatory size.

Luteinizing Hormone

Luteinizing hormone concentrations were analyzed beginning five days prior to treatment in order to obtain baseline values and continuing until three days post-ovulation. Similar to the results of James et al. (1997), there was not a significant difference in LH concentrations but a trend did exist such that the control mares demonstrated higher LH concentration during the test period compared to the altrenogest treated group. As demonstrated by Figure 4, the LH curve for the control mares resembles a normal LH profile whereas the curve

for the altrenogest treated mares was broader and the altrenogest treated mares demonstrated diminished amplitude in their LH peak. This supports the hypothesis that altrenogest administration during the follicular phase might alter the preovulatory surge of LH. Squires et al. (1983) and Lofstedt and Patel (1989) reported that LH is only slightly suppressed by altrenogest. Bruemmer et al. (2000) demonstrated a difference between treated and control groups in the amplitude of the LH peak. Their study administered twice the recommended dose of Regumate® and that may account for the significant difference. Wiepz et al. (1988) demonstrated that altrenogest can suppress or lower LH concentration. Figure 3 shows the effect of altrenogest on LH levels. Although not significant, there is a decrease in LH seen during the two days of altrenogest administration for the treated mares.

Progesterone

Progesterone concentrations were analyzed daily beginning five days prior to treatment for baseline values, and continuing until three days post-ovulation in an attempt to note differences in subsequent luteal function. There were no differences in P concentrations between altrenogest and control mares. This agrees with Bruemmer et al. (2000) in which no difference was observed in progesterone concentrations even when doubling the recommended dose of altrenogest. There was a decline in P for the altrenogest treated mares on the second day of treatment and on the subsequent day. This decline corresponds with the high concentration of estradiol exhibited in two of the treated mares.

When P concentration is low (as they are at this point of the estrous cycle) estradiol concentration is high and predominates.

Estradiol

Estradiol concentrations were also analyzed daily beginning five days prior to treatment for baseline values, and continuing until three days post-ovulation to evaluate the inhibitory effect of an exogenous progestin on follicular steroid secretions. Estradiol secretion is at its highest in the absence of progesterone (concentrations <1ng/mL). Estradiol concentrations increase progressively six to eight days before ovulation (at the beginning of the follicular phase or estrus). Follicular estradiol-17β and estrone sulfate peak one to two days prior to ovulation. It is the surge of estrogens that initiates LH release. At ovulation estradiol concentrations have been decreasing and will reach baseline diestrus levels near the end of estrus (within two days post-ovulation). Both groups in this study follow this pattern of secretion. Both groups seem to have a peak one to two days prior to ovulation. Also, both groups demonstrated a decline in estradiol beginning one day after ovulation which coincides with the rise in progesterone. Therefore, there appears to be no inhibitory effect of an exogenous progestin on estradiol secretion.

Pregnancy Rates

Pregnancy rates were analyzed to determine whether treatment with altrenogest to delay the time of ovulation would be detrimental to viability of the oocyte. Pregnancy rates for altrenogest treated and control mares were 85%

and 77% respectively. There was no significant difference in pregnancy rates between the two groups. This supports previous reports by James et al. (1997) and Bruemmer et al. (2000) that fertility was not affected by short-term altrenogest administration during the follicular phase.

Ultrasonographic examination was conducted on d 14 and d 16 post-ovulation to determine whether altrenogest administration had any detrimental effects on early embryonic development. The average size of the embryonic vesicle calculated with the available data for d 14 and d 16 in altrenogest treated versus control mares were 15.33 ± 4.80, 25.8 ± 2.77 and 15.00 ± 3.16, 25.17 ± 2.40, respectively. Short-term administration of altrenogest appears to have no detrimental effects on pregnancy rates or on early embryonic development.

CHAPTER VI

CONCLUSIONS

The efficacy of short term altrenogest administration in delaying ovulation was evaluated using twenty-six mares of light body type that ranged from three to 23 years of age. The hypothesis tested was that mares treated with altrenogest for two days would exhibit a delay in ovulation versus control mares. Mares were allocated to one of four stallions and then randomly assigned to either a treatment or control group. Treated mares received two consecutive oral doses of Regumate® once a 35 mm or greater follicle had developed. Control mares received two oral doses of Neobee M-5 oil. Blood samples were drawn daily beginning with the estrous cycle preceding the treated cycle. During the treated cycle, blood was collected daily with the following exception; blood was collected twice a day starting on the first day of altrenogest or oil administration and continuing until three days post-ovulation. In addition to analyzing days to ovulation, hormone concentrations (LH, progesterone and estradiol) and pregnancy rates were evaluated.

Days to ovulation for mares treated with altrenogest versus mares in the control group was longer ($P<0.05$). Mean days to ovulation for altrenogest treated mares was 6.10 ± 3.41. Mean days to ovulation for the control mares was 3.23 ± 2.00.

Luteinizing hormone, progesterone and estradiol concentrations were not statistically different between altrenogest treated and control mares (P=0.27, P=0.56, P=0.67). Therefore, hormone concentrations were not significantly affected by treatment. However, there was a trend for those mares treated with altrenogest to have an overall lower LH concentration and a diminished peak as compared to control mares. The expected result of altrenogest treated mares exhibiting a delay in their LH peak versus controls was not seen.

Evaluation of pregnancy rates is important to provide credibility before this treatment regimen is accepted for use by equine breeding facilities. Pregnancy rates were not different (P>0.05) between altrenogest treated (85%) and control groups (77%). Therefore, reproductive efficiency was not affected by treatment. Further, embryonic development was not detrimentally affected by treatment.

An important aspect of breeding management involves the determination of when ovulation will occur. This determination is necessary for artificially inseminating as close to the time of ovulation as possible while minimizing the number of inseminations per cycle. Although this study did provide evidence to conclude that short-term altrenogest administration does delay ovulation of a mature follicle, the treatment regimen requires further study. A field study utilizing larger numbers of mares in a variety of commercial breeding facilities would provide further credibility for a treatment regimen using Regumate® during estrus to delay ovulation.

LITERATURE CITED

Alexander, B.M., W.J. Murdoch, E.A. Vankirk and G.E. Moss. 1995. Attenuation of the estradiol-induced surge release of LH by antihistamine in ovariectomized ewes. Anim. Reprod. Sci. 40: 281-289.

Allen, W.R., S. Mathias, S.N. Lennard and R.E.S. Greenwood. 1995. Serial measurement of peripheral oestrogen and progesterone concentrations in oestrous mares to determine optimum mating time and diagnose ovulation. Equine Vet. J. 27(6): 460-464.

Allen, W.R., V. Urwin, D.J. Simson, R.E.S. Greenwood, R.C. Crowhurst, D.R. Ellis, S.W. Ricketts, M.D.N. Hunt and N.J.W. Digby. 1980. Preliminary studies on the use of an oral progestagen to induce oestrus and ovulation in seasonally anoestrous thoroughbred mares. Equine Vet. J., 12: 141-145.

Aurich, C., P.F. Daels, B. A. Ball, and J.E. Aurich. 1995. Effects of gonadal steroids on the opioid regulation of LH and prolactin release in ovariectomized pony mares. J. Endo. 147: 195-202.

Barraclough, C.A., and P.M. Wise. 1982. The role of catecholamines in the regulation of pituitary luteinizing hormone and follicle stimulating hormone secretion. Endocr. Rev., 3: 91-119.

Behrens, C., J.E. Aurich, E. Klug, H. Naumann, and H.O. Hoppen. 1993. Inhibition of gonadotropin release in mares during the luteal phase of the oestrous cycle by endogenous opioids. J. Reprod. Fert. 98: 509-514.

Blanchard, T.L., D.D. Varner, and J. Schumacher. 1998. Manual of Equine Reproduction. Mosby-Year Book, Inc. St. Louis, Missouri.

Bruemmer, J.E., R.C. Coy, A. Olson and E.L. Squires. 2000. Efficacy of altrenogest administration to postpone ovulation and subsequent fertility in mares. Equine Vet. J., 20: (7) 450-453.

Caldani, M., A. Caraty, J. Pelletier, J-C. Thiery and Y. Tillet. 1993. LH pulsatile release and its control. Pages 79-96 in Reproduction in Mammals and Man. C. Thibault, M.C. Levasseur, and R.H.F. Hunter, eds, Ellipses, Paris, France.

Channing, C.P., A.C. Wentz, and G. Jones. 1978. Steroid secretion by monkey and human ovarian cell types in vivo and in vitro. In: R. Scholler (ed.) Symposium on the Ovary. Fresnes, France.

Daels, P.F., P.M. McCue, M.J. DeMoraes, and J.P. Hughes. 1996. Persistence of the luteal phase following ovulation during altrenogest treatment in mares. Therio. 46: 799-811.

Douglas, R.H., and O.J. Ginther. 1976. Effects of repeated daily injections of prostaglandin on ovaries in mares. Prostaglandins, 12: 881-894.

Evans, M. J., R.G. Loy, T.B. Taylor and S.P. Barrows. 1982. Effects of exogenous steroids on serum FSH and LH, and on follicular development in cyclic mares. J. Reprod. Fert., Suppl. 32: 205-212.

Fanjul, L.F., R. de Galarreta, and J.J.W. Hsueh. 1983. Progestin augmentation of gonadotropin-stimulated progesterone production by cultured rat granulosa cells. Endo. 112: 405-407.

Garcia, M.C., L.J. Freedman, and O.J. Ginther. 1979. Interaction of seasonal and ovarian factors in the regulation of LH and FSH secretion in the mare. J. Reprod. Fert., Suppl. 27: 103-111.

Garcia, M.C., and O.J. Ginther. 1978. Regulation of plasma LH by estradiol and progesterone in ovariectomized mares. Biol. Reprod., 19: 447-453.

Ginther, O.J. 1992. Reproductive Biology of the Mare: Basic and Applied Aspects. 2nd ed. Equiservices, Cross Plains, Wisconsin.

Greenstein, B. 1994. Endocrinology at a Glance. Blackwell Science Ltd, Berlin, Germany.

Guyton, A.C., and J.E. Hall. 1996. Textbook of Medical Physiology. W.B. Saunders Co, Philadelphia, Pennsylvania.

Hoshmand, A.R. 1988. Statistical Methods for Agricultural Sciences. Timber Press, Portland, Oregon.

Irvine, C.H.G., and S.L. Alexander. 1997. Patterns of secretion of GnRH, LH & FSH during the postovulatory period in mares: mechanisms prolonging the LH surge. J. Reprod Fert., 109: 263-271.

Jacobs, B.R., and R.G. Smith. 1980. Evidence for a receptor-like protein for progesterone in bovine ovarian cytosol. Endo. 106: 1276-1282.

Jacobs, B.R., S. Suchocki, and R.G. Smith. 1980. Evidence for human ovarian progesterone receptor. Am.J. Obstet. Gynecol., 138: 332-336.

James, A.N., M.M. Vogelsang, D.W. Forrest, and G.G. Stott. 1997. Efficacy of short term administration of altrenogest to postpone ovulation in mares. J. Equine Vet. Sci. 18:329-331.

Karsch, F.S. 1987. Central action of ovarian steroids in the feedback regulation of pulsatile secretion of luteinizing hormone. Annu. Rev. Physiol., 49: 365-382.

Knobil, E., and J. Neill. 1999. The Physiology of Reproduction. Academic Press, San Diego, Californina.

Kurosumi, K., and K. Inoue. 1986. Ultrastructure of anterior pituitary cells. D Ganten and D Pfaff (ed.). Current Topics in Neuroendocrinology (Vol. 7). Springer-Verlag, New York.

Lang, A.L., M.M. Vogelsang, and G.D. Potter. 1995. Circadian patterns of plasma LH and testosterone concentrations in stallions in summer and winter. Proc. 14th Equine Nutr. Symp.p. 284.

Lofstedt, R.M., and J.H. Patel. 1989. Evaluation of the ability of altrenogest to control the equine estrous cycle. J. Amer. Vet. Med. Assoc., 194: 361-364.

Loy, R.G., and S.M. Swan. 1966. Effects of exogenous progestogens on reproductive phenomena in mares. J. Anim. Sci., 25: 821-826.

Mahesh, V.B., and D.W. Brann. 1992. Interaction between ovarian and adrenal steroids in the regulation of gonadotropin secretion. J. Steroid Biochem. Molec. Biol., 41: 495-513.

Matteri, R.L., J.F. Roser, D.M. Baldwin, V. Lipovetsky, and H. Papkoff. 1987. Characterization of a monoclonal antibody which detects luteinizing hormone-releasing hormone from diverse mammalian species. Domest. Anim. Endocr. 4(3): 157-165.

McKinnon, A.O., and J.L. Voss. 1993. Equine Reproduction. Lea and Febiger, Philadelphia, Pennsylvania.

McNatty, K.P., A. Makris, C. DeGrazia, R. Osathanondh, and K.J. Ryan. 1980. Steroidogenesis by recombined follicular cells from the human ovary in vitro. J. Clin. Endocr. Metab., 51: 1286-1292.

Nanda, A.S., W.R. Ward, and H. Hobson. 1991. Opioid involvement in LH release during the negative feedback effects of oestradiol and progesterone in dairy cows. Repro. Fert. Dev., 3: 709-114.

NRC. 1989. Nutrient Requirements of Horses (5th Ed.). National Academy Press, Washington, District of Columbia.

Palmer, E. 1978. Control of the estrous cycle of the mare. J. Repro. Fert. 54: 495-505.

Palmer, E. 1979. Reproductive management of mares without detection of oestrus. J. Reprod. Fert. Suppl., 27: 263-270.

Samper, J.C. 2000. Equine Breeding Management and Artificial Insemination. W.B. Saunders Co, Philadelphia, Pennsylvania.

SAS. 1999. SAS/STAT User's Guide (Release 8). SAS Inst., Cary, North Carolina.

Schreiber, J.R., and G.F. Erickson. 1979. Progesterone receptor in the rat ovary: further characterization and localization in the granulosa cell. Steroids, 34: 459-469.

Sharp, D.C., and W.R. Grubaugh. 1987. Use of push-pull perfusion techniques in studies of gonadotropin-releasing hormone secretion in mares. J. Reprod. Fert., Suppl. 35: 289-296.

Shupnik, MA. 1996. Gonadotropin gene modulation by steroids and gonadotropin-releasing hormone. Biol. Repro., 54: 279-286.

Sirois, J., B.A. Ball, J.E. Fortune. 1989. Patterns of growth and regression of ovarian follicles during the oestrous cycle and after hemiovariectomy in mares. Equine Vet. J., Suppl. 8:43-48.

Squires, E.L., C.P. Hesseman, S.K. Webel, R.K., and J.L. Voss. 1983. Relationship of altrenogest to ovarian activity hormone concentrations and fertility of mares. J. Anim. Sci., 56: 901-910.

Squires, E.L., W.B. Stevens, D.E. McGlothlin, and B.W. Pickett. 1979. Effect of an oral progestin on the estrous cycle and fertility of mares. J. Anim. Sci., 49: 729-735.

Turner, D.D., M.C. Garcia, S.K. Webel, and O.J. Ginther. 1981. Influence of follicular size on the response of mares to allyl trenbolone given before the onset of the ovulatory season. Therio., 16: 73-84.

Turner, J.E., and C.H.G. Irvine. 1991. The effects of various gonadotropin-releasing hormone regimens on gonadotropins, follicular growth and ovulation in deeply anoestrous mares. J. Repro. Fert., Suppl. 44:213-225.

VanNiekerk, C.H., J.C. Morgenthal, and W.H. Gernecke. 1975. Relationship between the morphology of and progesterone production by the corpus luteum of the mare. J. Repro. Fert. Suppl., 23: 171-175.

Webel, S.K., and E.L. Squires. 1982. Control of the estrous cycle in mares with altrenogest. J. Repro. Fert., Suppl. 32: 193-198.

Wiepz, G.J., E.L. Squires, and P.L. Chapman. 1988. Effects of norgestomet, altrenogest, and/or estradiol on follicular and hormonal characteristics of late transitional mares. Therio., 30: 181.

APPENDIX

RAW DATA FOR DAYS UNTIL OVULATION, LH, PROGESTERONE, ESTRADIOL CONCENTRATION

mare	tx/age	d of ov	d of tx	LH	P	E	PG
Ashley	tx/13	-11	-5	0	9.2	3	P
Ashley	barren	-10	-4	0	9.5	3	
Ashley		-9	-3	0	11.6	4	
Ashley		-8	-2	0	10	6	
Ashley		-7	-1	0	8.3	6	
Ashley		-6	0	0	7.6	8	
Ashley		-5	1	0	5.5	12	
Ashley		-4	2	0	0.8	16	
Ashley		-3	3	0	0.4	23	
Ashley		-2	4	0.7	0.1	28	
Ashley		-1	5	2.5	0.05	39	
Ashley		0	6	3.5	0.05	31	
Ashley		1	7	4.4	0.5	30	
Ashley		2	8	5.0	1.9	33	
Ashley		3	9	1.7	4.4	26	
Babe	tx/20	-8	-5	3.5	0.9	27	NP
Babe	barren	-7	-4	3.4	0.4	17	
Babe		-6	-3	2.6	0.2	23	
Babe		-5	-2	2.9	0.07	20	
Babe		-4	-1	4.3	0.09	24	
Babe		-3	0	4.9	0.1	31	
Babe		-2	1	4.2	0.08	31	
Babe		-1	2	5.7	0.07	32	
Babe		0	3	5.9	0.2	25	
Babe		1	4	7.5	1.0	25	
Babe		2	5	5.4	2.7	32	
Babe		3	6	2.9	4.8	29	
Binky	tx/14	-12	-7	6.3	1.5	9	NP
Binky	barren	-11	-6	5.6	0.5	11	
Binky		-10	-5	5.7	1.2	6	
Binky		-9	-4	5.9	0.2	12	
Binky		-8	-3	6.2	0.1	17	
Binky		-7	-2	.	0.1	17	
Binky		-6	-1	4.9	0.1	23	
Binky		-5	0	5.5	0.1	34	
Binky		-4	1	6.2	0.1	37	
Binky		-3	2	7.6	0.1	37	
Binky		-2	3	6.8	0.2	45	
Binky		-1	4	7.5	1.5	40	
Binky		0	5	5.0	3.6	35	
Binky		1	6	4.7	9.0	33	
Binky		2	7	3.6	13	31	
CC	tx/11	-7	-3	0	12.1	23	
CC		-6	-2	1.4	1.2	13	

RAW DATA FOR DAYS UNTIL OVULATION, LH, PROGESTERONE, ESTRADIOL CONCENTRATION (CON'T)

mare	tx/age	d of ov	d of tx	LH	P	E	PG
CC		-5	-1	1.8	0.3	17	
CC		-4	0	2.8	0.1	29	
CC		-3	1	3.0	0.08	30	
CC		-2	2	3.5	0	32	
CC		-1	3	5.7	0.04	34	
CC		0	4	8.0	0.1	32	
CC		1	5	8.5	1.1	27	
CC		2	6	7.1	2.5	26	
CC		3	7	5.9	4.8	26	
CC		4	8	3.3	6.6	21	
CandyBar	c/5	-13	-5	0	0.04	7	PG
CandyBar		-12	-4	0.6	0.06	7	
CandyBar		-11	-3	0.2	0.02	5	
CandyBar		-10	-2	1.6	0.08	5	
CandyBar		-9	-1	1.1	0	6	
CandyBar		-8	0	0.9	0.03	7	
CandyBar		-7	1	2.0	0.06	7	
CandyBar		-6	2	2.1	0.09	10	
CandyBar		-5	3	2.6	0.04	9	
CandyBar		-4	4	3.4	0.1	13	
CandyBar		-3	5	4.6	0.09	20	
CandyBar		-2	6	6.5	0.08	21	
CandyBar		-1	7	8.7	0.07	15	
CandyBar		0	8	8.9	0.6	18	
CandyBar		1	9	7.5	3.0	14	
CandyBar		2	10	5.2	5.5	11	
CandyBar		3	11	3.5	8.8	12	
CandyBar		4	12	2.0	9.7	9	
Diane	c/11	-10	-5	0.6	12.7	9	PG
Diane		-9	-4	0.9	11.9	13	
Diane		-8	-3	1.6	1.7	8	
Diane		-7	-2	2.6	0.7	8	
Diane		-6	-1	.	0.3	8	
Diane		-5	0	3.5	0.2	11	
Diane		-4	1	3.8	1.7	10	
Diane		-3	2	4.1	0.06	16	
Diane		-2	3	5.2	0.05	21	
Diane		-1	4	6.4	0.08	19	
Diane		0	5	8.1	0.2	24	
Diane		1	6	9.5	1.2	17	
Diane		2	7	8.4	4.2	17	
Diane		3	8	7.4	11.2	16	
Dixie	c/18	-7	-5	6.8	0	0	
Dixie		-6	-4	6.9	0	0	NP

RAW DATA FOR DAYS UNTIL OVULATION, LH, PROGESTERONE, ESTRADIOL CONCENTRATION (CON'T)

mare	tx/age	d of ov	d of tx	LH	P	E	PG
Dixie		-5	-3	6.6	0	1	
Dixie		-4	-2	8.6	0	2	
Dixie		-3	-1	7.4	0	3	
Dixie		-2	0	8.2	0	4	
Dixie		-1	1	9.0	0	4	
Dixie		0	2	9.0	0.1	3	
Dixie		1	3	13.3	1.2	1	
Dixie		2	4	9.5	3.1	2	
Dixie		3	5	9.3	4.3	1	
Dixie		4	6	6.9	7.9	3	
FoxyLady	c/3	-2	0	1.5	0.04	4	
FoxyLady		-1	1	2.9	0.03	9	
FoxyLady		0	2	5.0	0.02	12	
FoxyLady		1	3	6.1	0.5	7	
FoxyLady		2	4	5.8	2.4	5	
FoxyLady		3	5	3.8	4.9	4	
FoxyLady		4	6	2.6	7.2	2	
FoxyLady		5	7	2.8	9.6	1	
Lady	tx/5	-10	-3	3.6	1.7	7	
Lady		-9	-2	3.5	0.6	11	
Lady		-8	-1	3.7	0.3	12	
Lady		-7	0	3.8	0.1	9	
Lady		-6	1	4.0	0.1	13	
Lady		-5	2	5.3	0.1	4	
Lady		-4	3	4.5	0.09	13	
Lady		-3	4	4.6	0.04	14	
Lady		-2	5	4.3	0.03	13	
Lady		-1	6	6.5	0.02	20	
Lady		0	7	10.1	0.03	18	
Lady		1	8	11.2	0.4	18	
Lady		2	9	11.3	2.4	15	
Lady		3	10	7.7	4.9	13	
Lady		4	11	6.3	7.9	14	
MM	tx/6	-9	-5	2.6	20.4	33	PG
MM		-8	-4	3.0	1.4	31	
MM		-7	-3	3.8	0.7	27	
MM		-6	-2	3.8	0.3	28	
MM		-5	-1	4.6	0.2	34	
MM		-4	0	5.5	0.1	29	
MM		-3	1	4.8	0.2	41	
MM		-2	2	7.3	0.1	45	
MM		-1	3	8.9	0.1	35	
MM		0	4	12.2	0.1	41	
MM		1	5	13.1	1.0	34	

RAW DATA FOR DAYS UNTIL OVULATION, LH, PROGESTERONE, ESTRADIOL CONCENTRATION (CON'T)

mare	tx/age	d of ov	d of tx	LH	P	E	PG
MM		2	6	12.9	3.0	32	
MM		3	7	9.6	6.2	39	
MM		4	8	4.5	7.6	29	
Mandy	c/11	-8	-5	3.5	2.6	31	PG
Mandy		-7	-4	2.5	0.7	30	
Mandy		-6	-3	2.8	0.5	28	
Mandy		-5	-2	5.0	0.5	35	
Mandy		-4	-1	6.1	0.3	30	
Mandy		-3	0	7.5	0.7	38	
Mandy		-2	1	8.5	0.9	43	
Mandy		-1	2	9.6	1.0	48	
Mandy		0	3	11.6	1.3	48	
Mandy		1	4	9.5	1.4	40	
Mandy		2	5	10.8	3.9	37	
Mandy		3	6	11.5	7.5	34	
Mandy		4	7	6.1	11.4	32	
MariaFox	tx/3	-12	-5	.	0.05	0	PG
MariaFox		-11	-4	0	0.02	0	
MariaFox		-10	-3	0	0.07	0	
MariaFox		-9	-2	4.0	0.3	0	
MariaFox		-8	-1	0.4	0.03	0	
MariaFox		-7	0	0.8	0.05	0	
MariaFox		-6	1	4.0	0.4	0	
MariaFox		-5	2	4.0	0.6	0	
MariaFox		-4	3	4.0	1.0	0	
MariaFox		-3	4	1.8	0.1	2	
MariaFox		-2	5	1.9	0	4	
MariaFox		-1	6	5.1	0.02	4	
MariaFox		0	7	5.0	0.3	6	
MariaFox		1	8	4.7	1.1	7	
MariaFox		2	9	2.7	2.8	12	
MariaFox		3	10	1.0	3.4	7	
MariaFox		4	11	1.9	5.0	4	
Mercury	c/4	-8	-5	7.1	0.1	6	PG
Mercury		-7	-4	9.8	0.09	4	
Mercury		-6	-3	12.8	0.05	5	
Mercury		-5	-2	16.5	0.08	7	
Mercury		-4	-1	15.8	0.05	9	
Mercury		-3	0	16.9	0.8	11	
Mercury		-2	1	20	1.9	11	
Mercury		-1	2	17.2	2.8	11	
Mercury		0	3	13.7	5.0	7	
Mercury		1	4	18.4	6.9	10	
Mercury		2	5	7.1	8.2	6	

RAW DATA FOR DAYS UNTIL OVULATION, LH, PROGESTERONE, ESTRADIOL CONCENTRATION (CON'T)

mare	tx/age	d of ov	d of tx	LH	P	E	PG
Mercury		3	6	7.2	10.9	10	
Merrily	c/5	-8	-5	1.2	7.7	317	PG
Merrily		-7	-4	0.4	7.6	375	
Merrily		-6	-3	0.8	10.9	430	
Merrily		-5	-2	1.9	9.3	389	
Merrily		-4	-1	1.4	0.9	444	
Merrily		-3	0	3.1	0.2	276	
Merrily		-2	1	5.6	0.1	374	
Merrily		-1	2	8.6	0	390	
Merrily		0	3	10.7	0	352	
Merrily		1	4	13.7	0.7	304	
Merrily		2	5	13	2.8	261	
Merrily		3	6	10.4	5.0	265	
Merrily		4	7	9.2	5.9	259	
MsOakly	c/3	-6	-5	2.1	2.2	9	NP
MsOakly		-5	-4	1.8	0.3	8	
MsOakly		-4	-3	5.5	0.1	6	
MsOakly		-3	-2	6.3	0.2	8	
MsOakly		-2	-1	7.4	0.07	12	
MsOakly		-1	0	10	0.04	10	
MsOakly		0	1	15	0.1	8	
MsOakly		1	2	17.8	1.1	8	
MsOakly		2	3	18.1	3.2	7	
MsOakly		3	4	17.3	3.6	7	
NBM	tx/3	-10	-5	2.2	0.3	6	PG
NBM		-9	-4	2.0	0.1	10	
NBM		-8	-3	1.5	0	7	
NBM		-7	-2	3.0	0.02	7	
NBM		-6	-1	1.8	0	10	
NBM		-5	0	2.3	0	16	
NBM		-4	1	0.9	0.04	18	
NBM		-3	2	1.4	0	17	
NBM		-2	3	3.6	0	27	
NBM		-1	4	5.1	0	30	
NBM		0	5	6.9	0.03	36	
NBM		1	6	7.5	0.9	33	
NBM		2	7	4.9	3.0	19	
NBM		3	8	2.2	5.3	17	
NBM		4	9	6.3	7.0	15	
NuEnt	c/3	-6	-4	2.3	0.06	5	PG
NuEnt		-5	-3	2.3	0.07	8	
NuEnt		-4	-2	.	.	.	
NuEnt		-3	-1	.	.	.	
NuEnt		-2	0	5.5	0.03	23	

RAW DATA FOR DAYS UNTIL OVULATION, LH, PROGESTERONE, ESTRADIOL CONCENTRATION (CON'T)

mare	tx/age	d of ov	d of tx	LH	P	E	PG
NuEnt		-1	1	10.6	0.09	19	
NuEnt		0	2	11.2	0.4	23	
NuEnt		1	3	13.0	2.0	20	
NuEnt		2	4	5.3	4.7	15	
NuEnt		3	5	3.5	8.8	11	
NuEnt		4	6	0.9	9.5	12	
NNN	tx/4	-17	-5	0.9	0.04	611	PG
NNN		-16	-4	1.8	0	573	
NNN		-15	-3	1.5	0	541	
NNN		-14	-2	1.9	0	504	
NNN		-13	-1	1.4	0	643	
NNN		-12	0	1.6	0	639	
NNN		-11	1	2.9	0	617	
NNN		-10	2	4.5	0	551	
NNN		-9	3	3.4	0.03	502	
NNN		-8	4	4.0	0	487	
NNN		-7	5	3.8	0	442	
NNN		-6	6	3.7	0.8	437	
NNN		-5	7	2.2	0.02	420	
NNN		-4	8	3.0	0	399	
NNN		-3	9	2.4	0	422	
NNN		-2	10	3.4	0	438	
NNN		-1	11	4.5	0	308	
NNN		0	12	5.8	0.2	377	
NNN		1	13	7.8	1.9	344	
NNN		2	14	7.5	4.7	314	
NNN		3	15	5.6	7.8	287	
NNN		4	16	3.5	10	297	
NuRey	c/4	-8	-5	4.0	0.1	698	PG
NuRey		-7	-4	2.2	0	627	
NuRey		-6	-3	3.0	0.5	749	
NuRey		-5	-2	2.2	0.1	638	
NuRey		-4	-1	2.2	0.1	619	
NuRey		-3	0	2.8	0	522	
NuRey		-2	1	3.2	0.2	603	
NuRey		-1	2	5.7	0	480	
NuRey		0	3	6.3	0.05	443	
NuRey		1	4	8.2	0.9	472	
NuRey		2	5	7.7	2.6	429	
NuRey		3	6	6.7	4.2	406	
NuRey		4	7	.	5.0	408	
PCPuff	tx/4	-7	-5	0.9	0.06	1720	PG
PCPuff		-6	-4	0.4	0.08	1518	
PCPuff		-5	-3	0.5	0.1	1243	

RAW DATA FOR DAYS UNTIL OVULATION, LH, PROGESTERONE, ESTRADIOL CONCENTRATION (CON'T)

mare	tx/age	d of ov	d of tx	LH	P	E	PG
PCPuff		-4	-2	1.1	0.02	1310	
PCPuff		-3	-1	1.9	0.1	1268	
PCPuff		-2	0	1.9	0.02	1346	
PCPuff		-1	1	2.1	0	1373	
PCPuff		0	2	1.9	0	1188	
PCPuff		1	3	3.2	0.2	1170	
PCPuff		2	4	2.8	1.3	1139	
PCPuff		3	5	2.9	2.4	997	
PCPuff		4	6	3.6	4.7	1000	
PCPuff		5	7	2.9	4.8	972	
Polly	c/23	-8	-5	1.4	14.4	18	NP
Polly		-7	-4	4.5	2.9	23	
Polly		-6	-3	3.5	0.5	23	
Polly		-5	-2	3.3	0.1	21	
Polly		-4	-1	3.5	0.1	31	
Polly		-3	0	6.5	0.1	32	
Polly		-2	1	8.9	0.1	45	
Polly		-1	2	10.9	0.09	37	
Polly		0	3	13.0	0.07	33	
Polly		1	4	12.0	1.1	23	
Polly		2	5	13.3	2.5	20	
Polly		3	6	10.3	5.6	24	
Skippy	c/6	-5	-5	7.7	0.2	8	PG
Skippy		-4	-4	6.4	0.1	5	
Skippy		-3	-3	9.2	0.07	4	
Skippy		-2	-2	9.3	0.04	7	
Skippy		-1	-1	10.4	0.04	9	
Skippy		0	0	12.6	0.04	6	
Skippy		1	1	15.2	0.4	4	
Skippy		2	2	16.5	1.7	4	
Skippy		3	3	20.2	3.1	3	
Skippy		4	4	16.2	3.7	4	
SnipaBar	tx/5	-9	-5	1.8	0.05	8	PG
SnipaBar		-8	-4	2.2	0	5	
SnipaBar		-7	-3	3.5	0	6	
SnipaBar		-6	-2	3.3	0	7	
SnipaBar		-5	-1	3.2	0	8	
SnipaBar		-4	0	3.5	0	15	
SnipaBar		-3	1	3.4	0	17	
SnipaBar		-2	2	3.9	0.02	29	
SnipaBar		-1	3	6.0	0	27	
SnipaBar		0	4	7.5	0.1	32	
SnipaBar		1	5	9.2	1.1	23	
SnipaBar		2	6	5.6	2.9	21	

RAW DATA FOR DAYS UNTIL OVULATION, LH, PROGESTERONE, ESTRADIOL CONCENTRATION (CON'T)

mare	tx/age	d of ov	d of tx	LH	P	E	PG
SnipaBar		3	7	4.2	4.9	19	
SnipaBar		4	8	3.3	6.7	14	
SnipaBar		5	9	5.7	8.6	16	
Sparticus	tx/18	-9	-5	5.9	1.5	36	PG
Sparticus		-8	-4	6.2	2.0	36	
Sparticus		-7	-3	5.5	3.1	24	
Sparticus		-6	-2	5.0	2.8	28	
Sparticus		-5	-1	3.0	2.8	34	
Sparticus		-4	0	3.0	3.1	28	
Sparticus		-3	1	5.0	1.6	26	
Sparticus		-2	2	5.4	0.3	33	
Sparticus		-1	3	5.8	0.04	27	
Sparticus		0	4	9.2	0.09	32	
Sparticus		1	5	9.1	0.5	18	
Sparticus		2	6	10	1.6	21	
Sparticus		3	7	8.1	2.7	22	
Starlet	c/3	-10	-5	0.5	0	0	PG
Starlet		-9	-4	0.7	0	0	
Starlet		-8	-3	1.0	0	0	
Starlet		-7	-2	0.9	0	0	
Starlet		-6	-1	1.0	0	0	
Starlet		-5	0	1.8	0	0	
Starlet		-4	1	1.7	0	4	
Starlet		-3	2	2.4	0	8	
Starlet		-2	3	2.2	0	13	
Starlet		-1	4	3.2	0	19	
Starlet		0	5	5.7	0	21	
Starlet		1	6	4.7	0.1	20	
Starlet		2	7	5.3	1.9	15	
Starlet		3	8	1.0	3.7	11	
Starlet		4	9	2.8	7.7	9	
ThatsFoxy	tx/3	-19	-5	1.1	0.06	0	PG
ThatsFoxy		-18	-4	1.4	0	0	
ThatsFoxy		-17	-3	1.2	0.02	0	
ThatsFoxy		-16	-2	1.3	0.04	0	
ThatsFoxy		-15	-1	1.1	0	1	
ThatsFoxy		-14	0	1.0	0.05	9	
ThatsFoxy		-13	1	0.9	0	5	
ThatsFoxy		-12	2	2.9	0.08	3	
ThatsFoxy		-11	3	3.4	0	1	
ThatsFoxy		-10	4	3.3	0.05	3	
ThatsFoxy		-9	5	3.0	0	2	
ThatsFoxy		-8	6	6.5	0	2	
ThatsFoxy		-7	7	4.1	0	2	

RAW DATA FOR DAYS UNTIL OVULATION, LH, PROGESTERONE, ESTRADIOL CONCENTRATION (CON'T)

mare	tx/age	d of ov	d of tx	LH	P	E	PG
ThatsFoxy		-6	8	4.3	0.02	6	
ThatsFoxy		-5	9	2.9	0	12	
ThatsFoxy		-4	10	2.8	0	8	
ThatsFoxy		-3	11	2.8	0	25	
ThatsFoxy		-2	12	4.3	0	32	
ThatsFoxy		-1	13	5.2	0	29	
ThatsFoxy		0	14	6.3	0.4	24	
ThatsFoxy		1	15	4.7	2.3	22	
ThatsFoxy		2	16	2.0	5.1	21	
ThatsFoxy		3	17	1.9	7.2	16	
ThatsFoxy		4	18	5.6	9.3	16	
ThatsFoxy		5	19	10.8	10.4	11	

RAW DATA FOR SIZE OF FOLLICLE AT OVULATION

mare	tx	Follicle (mm)
Ashley	tx	46
Babe	tx	48
Binky	tx	50
CandyBar	c	52
CC	tx	46
Diane	c	57
Dixie	c	41
FoxyLady	c	41
Lady	tx	52
MM	tx	48
Mandy	c	45
MariaFox	tx	43
Mercury	c	47
Merrily	c	48
MsOakly	c	37
NBM	tx	42
NuEnt	c	45
NNN	tx	50
NuRey	c	45
PCPuff	tx	40
Polly	c	40
Skippy	c	35
SnipaBar	tx	47
Sparticus	tx	38
Starlet	c	49
ThatsFoxy	tx	45

ANOVA TABLES FOR DAYS TO OVULATION

Number of Days to Ovulation

Dependent Varible: dtov

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	25	248.7788465	9.9511538	.	.
Error	0	0.00000000	.		
Corrected Total	25	248.7788462			

R-Square	Coeff Var	Root MSE	Dtov Mean
1.000000	.	.	4.634615

Source	DF	Type 1 SS	Mean Square	F value	Pr > F
trt	1	57.0096154	57.0096154	.	.
horse (trt)	24	191.7692308	7.9903846	.	.

Source	DF	Type III SS	Mean Square	F value	Pr > F
trt	1	57.0096154	57.0096154	.	.
horse (trt)	24	191.7692308	7.99003846	.	.

Tests of Hypotheses Using the Type III MS for horse (trt) as an Error Term

Source		Type III SS	Mean Square	F value	Pr > F
trt	1	57.0096154	57.0096154	7.13	0.0134
horse (trt)	24	191.7692308	7.9903846	1.0	0.5000

ANOVA TABLES FOR SIZE OF FOLLICLE AT OVULATION

Size of Follicle before Ovulation

Dependent Varible: follicle

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	25	651.1153846	26.0446154	.	.
Error	0	0.00000000	.		
Corrected Total	25	651.1153846			

R-Square	Coeff Var	Root MSE	follicl Mean
1.000000	.	.	45.26923

Source	DF	Type 1 SS	Mean Square	F value	Pr > F
trt	1	6.5000000	6.5000000	.	.
horse (trt)	24	644.6153846	26.8589744	.	.

Source	DF	Type III SS	Mean Square	F value	Pr > F
trt	1	6.5000000	6.5000000	.	.
horse (trt)	24	644.6153846	26.8589744	.	.

Tests of Hypotheses Using the Type III MS for horse (trt) as an Error Term

Source		Type III SS	Mean Square	F value	Pr > F
trt	1	6.5000000	6.5000000	0.24	0.6272
horse (trt)	24	644.6153846	26.8589744	1.00	0.5000

ANOVA TABLES FOR LH CONCENTRATION

LH Concentration with Day 0 as Ovulation

Dependent Varible: LH

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	67	4484.982447	66.940037	15.62	<.0001
Error	290	1242.542609	4.284630		
Corrected Total	357	5727.525056			

R-Square	Coeff Var	Root MSE	LH Mean
0.783058	39.59163	2.069935	5.228212

Source	DF	Type 1 SS	Mean Square	F value	Pr > F
trt	1	610.454742	610.454742	142.48	<.0001
horse (trt)	24	2231.142907	92.964288	21.70	<.0001
day	24	1486.346156	61.931090	14.45	<.0001
trt*day	18	157.038642	8.724369	2.04	0.0083

Source	DF	Type III SS	Mean Square	F value	Pr > F
trt	1	103.783707	103.783707	24.22	<.0001
horse (trt)	24	1941.386862	80.891119	18.88	<.0001
day	24	1536.927788	64.038658	14.95	<.0001
trt*day	18	157.038642	8.724369	2.04	0.0083

Tests of Hypotheses Using the Type III MS for horse (trt) as an Error Term

Source		Type III SS	Mean Square	F value	Pr > F
trt	1	103.7837074	103.7837074	1.28	0.2685
trt*day	18	157.0386419	8.7243690	0.11	1.0000

ANOVA TABLES FOR P CONCENTRATION

Progesterone Concentration with Day 0 as Ovulation

Dependent Varible: P

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	67	2070.381761	30.901220	5.52	<.0001
Error	294	1645.2677623	5.596148		
Corrected Total	361	3715.649385			

R-Square	Coeff Var	Root MSE	P Mean
0.557206	123.9781	2.365618	1.908094

Source	DF	Type 1 SS	Mean Square	F value	Pr > F
trt	1	12.800105	12.800105	2.29	0.1315
horse (trt)	24	364.075304	15.169804	2.71	<.0001
day	24	1651.896295	68.829012	12.30	<.0001
trt*day	18	41.610057	2.311670	0.41	0.9847

Source	DF	Type III SS	Mean Square	F value	Pr > F
trt	1	6.080700	6.080700	1.09	0.2981
horse (trt)	24	419.456075	17.477336	3.12	<.0001
day	24	1609.232593	67.051358	11.98	<.0001
trt*day	18	41.610057	2.311670	0.41	0.9847

Tests of Hypotheses Using the Type III MS for horse (trt) as an Error Term

Source		Type III SS	Mean Square	F value	Pr > F
trt	1	6.08069969	6.08069969	0.35	0.5608
trt*day	18	41.61005722	2.31166985	0.13	1.0000

ANOVA TABLES FOR E CONCENTRATION

Estradiol Concentration with Day 0 as Ovulation

Dependent Varible: E

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	67	25575320.37	381721.20	126.62	<.0001
Error	295	886291.70	3014.60		
Corrected Total	361	26461612.07			

R-Square	Coeff Var	Root MSE	E Mean
0.966507	46.42671	54.90535	118.2624

Source	DF	Type 1 SS	Mean Square	F value	Pr > F
trt	1	341580.57	341580.57	113.31	<.0001
horse (trt)	24	25071433.42	1044643.06	346.53	<.0001
day	24	138661.44	5777.56	1.92	0.0070
trt*day	18	23644.94	1313.61	0.44	0.9794

Source	DF	Type III SS	Mean Square	F value	Pr > F
trt	1	196362.04	196362.04	65.14	<.0001
horse (trt)	24	24530438.19	1022101.59	339.05	<.0001
day	24	108539.07	4522.46	1.50	0.0653
trt*day	18	23644.94	1313.61	0.44	0.9794

Tests of Hypotheses Using the Type III MS for horse (trt) as an Error Term

Source		Type III SS	Mean Square	F value	Pr > F
trt	1	196362.0428	196362.0428	0.19	0.6868
trt*day	18	23644.9406	1313.6078	0.00	1.0000

ANOVA TABLES FOR LH CONCENTRATION

LH Concentration with Day 0 as Day of Treatment

Dependent Varible: LH

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	66	4483.395983	67.930242	14.93	<.0001
Error	291	1324.467816	4.551436		
Corrected Total	357	5807.863799			

R-Square	Coeff Var	Root MSE	LH Mean
0.771953	41.10660	2.133409	5.189944

Source	DF	Type 1 SS	Mean Square	F value	Pr > F
trt	1	642.486122	642.486122	141.16	<.0001
horse (trt)	24	2282.844645	95.118527	20.90	<.0001
day	24	1294.957130	53.956547	11.85	<.0001
trt*day	17	263.108086	15.476946	3.40	<.0001

Source	DF	Type III SS	Mean Square	F value	Pr > F
trt	1	351.502927	351.502927	77.23	<.0001
horse (trt)	24	2533.260802	105.5522533	23.19	<.0001
day	24	1282.950317	53.456263	11.74	<.0001
trt*day	17	263.108086	15.476946	3.40	<.0001

Tests of Hypotheses Using the Type III MS for horse (trt) as an Error Term

Source		Type III SS	Mean Square	F value	Pr > F
trt	1	351.5029267	351.5029267	3.33	0.0805
trt*day	17	263.1080855	15.4769462	0.15	0.9999

ANOVA TABLES FOR P CONCENTRATION

Progesterone Concentration with Day 0 as Day of Treatment

Dependent Varible: P

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	66	1735.735661	26.299025	3.9	<.0001
Error	295	1987.066343	6.735818		
Corrected Total	361	3722.802004			

R-Square	Coeff Var	Root MSE	P Mean
0.466244	136.4721	2.595345	1.901740

Source	DF	Type 1 SS	Mean Square	F value	Pr > F
trt	1	13.577079	13.577079	2.02	0.1567
horse (trt)	24	368.248361	15.343682	2.28	0.0008
day	24	1113.355798	46.389825	6.89	<.0001
trt*day	17	240.554423	14.150260	2.10	0.0071

Source	DF	Type III SS	Mean Square	F value	Pr > F
trt	1	172.585042	172.585042	25.62	<.0001
horse (trt)	24	588.729404	24.530392	3.64	<.0001
day	24	1158.985375	48.291057	7.17	<.0001
trt*day	17	240.554423	14.150260	2.10	0.0071

Tests of Hypotheses Using the Type III MS for horse (trt) as an Error Term

Source		Type III SS	Mean Square	F value	Pr > F
trt	1	172.5850420	172.5850420	7.04	0.0739
trt*day	17	240.5544231	14.1502602	0.58	0.8772

ANOVA TABLES FOR E CONCENTRATION

Estradiol Concentration with Day 0 as Day of Treatment

Dependent Varible: E

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	66	25564153.35	387335.66	127.39	<.0001
Error	295	896987.66	3040.64		
Corrected Total	361	26461141.01			

R-Square	Coeff Var	Root MSE	E Mean
0.966102	46.62460	55.14196	118.2680

Source	DF	Type 1 SS	Mean Square	F value	Pr > F
trt	1	341689.32	341689.32	112.37	<.0001
horse (trt)	24	25070862.66	1044619.28	343.55	<.0001
day	24	128573.38	5357.22	1.76	0.0168
trt*day	17	23027.99	1354.59	0.45	0.9732

Source	DF	Type III SS	Mean Square	F value	Pr > F
trt	1	172279.45	172279.45	56.66	<.0001
horse (trt)	24	24827702.89	1034487.62	340.22	<.0001
day	24	119726.91	4988.62	1.64	<.0001
trt*day	17	23027.99	1354.59	0.45	0.0071

Tests of Hypotheses Using the Type III MS for horse (trt) as an Error Term

Source		Type III SS	Mean Square	F value	Pr > F
trt	1	172279.4518	172279.4518	0.17	0.6868
trt*day	17	23027.9916	1354.5877	0.00	1.0000

CHI-SQUARE ANALYSIS OF PREGNANCY RATES

Treatment	Not Pregnant	Pregnant	Total Bred
Control	2	10	13
Treated	3	11	13
Total	5	21	26

f_e = (Row Total) (Column Total)/ (Grand Total)

Treatment	Not Pregnant	Pregnant
Control	2.5	10.5
Treated	2.5	10.5

f_0	f_e	$f_0 - f_e$	$(f_0 - f_e)^2$	$(f_0 - f_e)^2 / f_e$
2	2.5	-0.5	.25	0.1
11	10.5	.5	.25	0.023
3	2.5	-0.5	.25	0.1
10	10.5	.5	.25	0.023

$X^2 = \sum (f_0 - f_e)^2 / f_e$

$X^2 = 0.246$

Critical Value = 3.841

If computed (0.246) < Critical Value then accept null hypothesis concluding that pregnancy rates are the same.

VITA

Sandra “Sandy” Lee Murrell

24 Cedarfield Court
Palm Coast, Florida 32137

Texas A&M University, College Station, Texas

M.S., Physiology of Reproduction, Aug. 2003

B.S., Biomedical Science, Dec. 1993

Kirks Rockin K Ranch, Inc. – Celina, Texas (Oct. 2002 – present)
- Breeding manager

Colby Community College – Colby, Kansas (Aug 2001 – Aug 2002)
- Horse Production Director/Instructor/Horse Show Team Coach

Texas A&M University – College Station, Texas
- TAMU Introductory Biology Department
  - Teaching Assistant (Aug 1996 – May 2002)
    - Introductory Biology lab 123 (Aug 1996 – May 1998)
    - Introductory Biology lab 124 (Aug 1998 – May 2000)
    - Introductory Zoology lab 107 (Aug 2000 – May 2002)
- TAMU Horse Center – College Station, Texas (summer 1996, 1997)
  - Student Worker
- TAMU Department of Veterinary Pathobiology (Dec 1991 – Jan 1995)
  - Lab Technician
- TAMU Department of Animal Science
  - Instructor, TAMU Horse Breeder’s School Shortcourse

Buck’s Livery – Purgatory, Colorado (summer 1999)
- Wrangler

Wichita Ranch – Brenham, Texas (Jan 1995 – Aug 1995)
- Breeding Intern

Wellborn Road Veterinary Hospital – College Station, Texas (Aug 1994 – Dec 1995)
- Veterinary Technician

Dumfries Animal Hospital – Dumfries, Virginia (Dec 1993 – May 1994)
- Veterinary Technician